ABERRANT PHENOTYPES IN ACUTE MYELOID LEUKEMIA IN INDIA

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CERTIFICATE

This is to certify that the dissertation entitled **"ABERRANT PHENOTYPES IN ACUTE MYELOID LEUKEMIA IN INDIA"** is a bonafide work done by **DR.A.THELENGANA**, Post Graduate Student, Institute of Internal Medicine, Madras Medical College, Chennai-3, in partial fulfillment of the University Rules and Regulations for the award of MD Branch – I General Medicine, under our guidance and supervision, during the academic year 2012 - 2015.

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DECLARATION

I, **Dr.A.THELENGANA** solemnly declare that dissertation titled **"ABERRANT PHENOTYPES IN ACUTE MYELOID LEUKEMIA IN INDIA"** is a bonafide work done by me at Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3 during February 2014 to July 2014 under the guidance and supervision of my unit chief **Prof.R.PENCHALAIAH, M.D.,** Professor of Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. This dissertation is submitted to Tamilnadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (Branch – I) in General Medicine**

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ABREVIATIONS

AML	: ACUTE MYELOID LEUKEMIA
FCM	: FLOWCYTOMETRY
Ly +	: LYMPHOID ASSOCIATED ANTIGEN POSITIVE
APL	: ACUTE PROMYELOCYTIC LEUKEMIA
MPO	: MYELOPEROXIDASE
MDS	: MYELODYSPLASTIC SYNDROME
PAS	: PERIODIC ACID SCHIFF
ITD	: INTERNAL TANDEM DUPLICATION
FLT	: FMS LIKE TYROSINE
ATRA	: ALL TRANS RETINOIC ACID
NEC	: NON ERYTHROID CELLS
N:C	: NUCLEUS: CYTOPLASM
VMF	: VON WILLEBRAND FACTOR
FSC	: FORWARD SCATTERED LIGHT
SSC	: SIDE SCATTERED LIGHT
RAEB	: REFRACTORY ANAEMIA WITH ERYTHROID BLAST

HSCT	: HEMATOPOETIC STEM CELL TRANSFUSION
ECOG PS	: EASTERN COOPERATIVE ONCOLOGY GROUP PERFORMANCE STATUS
RARA	: RETINOIS ACID RECEPTOR ALPHA
PML	: PROMYELOCYTIC LEUKEMIA GENE
PLZF	: PROMYELOCYTIC LEUKEMIC ZINC FINGER
NUMA	: NUCLEAR MATRIX ASSOCIATED
NPM1	: NUCLEOPHOSMIN 1

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease, presenting with a high diversity of phenotypes . Immunophenotype in acute myeloid leukemia (AML) had remained elusive. In recent years, along with the wide application of AML immunophenotype testing, immunophenotype itself and its relationship with genetics and morphology became better understood .

The latest WHO 2008 classification of acute leukemia uses morphology, immunophenotype, genetics and clinical features to define clinically significant disease entities . Distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) is extremely important and flowcytometry (FCM) is very instrumental in this. Malignant blasts often have an abnormal phenotype that allows distinction from normal immature cells .

Aberrant phenotype is a well known phenomenon in acute myeloid leukemia. Currently, the aberrant phenotypes are classified into different types: co-expression

CONTENTS

ABSTRACT

Background: Aberrant phenotype has been reported in acute leukemias with varying frequency in various studies . Its prognostic importance still remains controversial. In acute myeloid leukemias, aberrant phenotype, as high as 88 %, has been reported. In the current study, 35 samples of newly diagnosed acute myeloid leukemia were analyzed to evaluate the frequency of aberrant phenotypes. Aberrant phenotypes were also correlated with known prognostic factors such as gender, age, WBC count, platelet count and blast percentage.

Materials and Methods: Whole blood or bone marrow aspirate collected in EDTA were processed by standard method and subjected to flowcytometric immunophenotyping for marker CD3, CD7, CD10 ,CD13, CD14 ,CD15, CD19 CD33, CD34 and CD117.

Results: Aberrant lymphoid markers were seen in 17(49%)cases . 5 cases had lymphoid associated antigen expression alone which is 14 % of cases. 3 cases had asynchronous antigen expression alone which is 8% of cases. 9 cases had both asynchronous antigen expression and lymphoid associated antigen expression which is 27 % of cases . In total lymphoid associated antigen expression is seen in 41 % of cases and asynchronous antigen expression in 35 % of cases. CD3 , CD19 (lymphoid associated antigen) and CD34+ CD15+ (asynchronous aberrant

phenotype) were the most common equally expressed aberrant phenotypes, each 7 cases. CD 7 was seen in 4 cases , 28.6% of Ly + AML aberrant phenotypic cases. CD 3 was significantly more common in males (P=0.021) but in general there were no statistically significant association between poor prognostic factors and aberrant phenotypic AML.

Conclusion: There is a frequent occurrence of aberrant phenotype in acute myeloid leukemia in India like in other majority of studies . CD19 and CD3 were the most commonly expressed lymphoid associated antigen. Lymphoid associated expression were slightly more common than asynchronous antigen expression. Most common asynchronous aberrant phenotype was CD34+CD15+. Aberrant phenotypic expression were not associated with poor risk factors in acute myeloid leukemia except for common expression of CD3 in males.

Key words: Aberrant phenotype; Flow cytometry; AML ;Prognosis ; Lymphoid associated antigen ; asynchronous antigen.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease, presenting with a high diversity of phenotypes . Immunophenotype in acute myeloid leukemia (AML) had remained elusive. In recent years, along with the wide application of AML immunophenotype testing, immunophenotype itself and its relationship with genetics and morphology became better understood .

The latest WHO 2008 classification of acute leukemia uses morphology, immunophenotype, genetics and clinical features to define clinically significant disease entities . Distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) is extremely important and flowcytometry (FCM) is very instrumental in this. Malignant blasts often have an abnormal phenotype that allows distinction from normal immature cells .

Aberrant phenotype is a well known phenomenon in acute myeloid leukemia. Currently, the aberrant phenotypes are classified into different types: co-expression of lymphoid-associated antigens or lineage infidelity; asynchronous antigen expression, in which early antigens are coexpressed with more mature ones; or

antigen overexpression and existence of abnormal light scatter patterns.

Aberrant antigen expression is reported to have variable frequency . The most frequent lymphoid antigens in AML that have been reported include, CD7 (T-cell marker) and CD19 (B-cell marker). In AML, characteristic antigens have been related to particular morphological FAB subtypes and associated with the presence of recurrent genetic abnormalities such as AML-M2 with t(8;21) that shows aberrant expression of lymphoid markers include CD19 and CD56 another one is co-expression of CD2 in M4E with inv(16) or t(16; 16), although not specific for this type of AML and M5 with t(9;11) is reported to have high expression CD56.

One of the newly emerging importance of immunophenotypical aberrancies using FCM is the detection and quantification of minimal residual disease (MRD) for providing prognostic information, and make use of such aberrancies in routine management of patients to guide therapy . Prognostic value of aberrant phenotype and its association with adverse clinical, hematological, and other biological prognostic factors in acute myeloid leukemia is still controversial. In some studies, the aberrant phenotypes were found to be of prognostic significance while other studies report no difference between aberrant and normal phenotype. In the literature, Ly + AML phenotypes have been shown to be associated with both poor

as well as favorable prognosis .However, no difference has also been reported for the clinical features and outcome between normal and aberrant phenotype of childhood AML . Based on coexpression and correlation of lineage-associated antigens, multiparameter high-resolution flow cytometry has been developed to precisely identify lineage characteristics of leukemia. Immunophenotyping improves both accuracy and reproducibility of acute leukemia classification and is considered particularly useful for identifying poorly differentiated subtypes of acute leukemia, acute myeloid leukemia (AML) with lymphoid marker expression and acute lymphatic leukemia (ALL) with myeloidmarker expression. Immunological studies of leukemic blasts have become critical also for identifying biphenotypic and bilineal acute leukemias.

At present, while the prognostic value of individual antigen expressions is still controversial, it is important in the immunologic detection of minimal residual disease, especially in AML, as it seems to be important in monitoring the acute leukemia patients in remission There is much to be discovered about the characteristics and significance of co-expression of two or more aberrant lineage leukemia markers.

In the current study, 35 samples of newly diagnosed acute myeloid leukemia were analyzed to evaluate the frequency of aberrant phenotypes. Aberrant phenotypes were also correlated with known prognostic factors such as gender, age, WBC count, platelet count and blast percentage.

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AIMS AND OBJECTIVES

- To study about the aberrant phenotypes in acute myeloid leukemia in India.
- To study the correlation among the aberrant phenotypes and poor prognostic factors in acute myeloid leukemia.

REVIEW OF LITERATURE

DEFINITION

Acute myelogenous leukemia is a clonal, malignant disease of hematopoietic tissue.

There is

(1) accumulation of myeloblasts mainly in the bone marrow and

(2) impairment of production of normal hematopoietic cells.

EPIDEMIOLOGY

Acute myeloid leukemia is common in neonates but less common in children and adolescents. Males are slightly more commonly involved than females. The mortality rate from acute myeloid leukemia is approximately 0.5 / lakh patients younger than 10 yrs. Mortality rate increases after that and reaches up to 20/lakh patients in their nineties.

POPULATION	INCIDENCE
ASIAN	LOWER
EASTERN EUROPEAN	HIGHER
SPANISH(APL)	HIGHER
DEVELOPED NATIONS	HIGHER
INDUSTRIALIZED CITIES	HIGHER

AML accounts for 80 to 90% of cases of acute leukemia in adults and in children about 15 to 20%. With increasing age the incidence increases , with a median of 67 years. For people less than 25 yrs. it is less than 1/100,000, but for octogenarians of it is 25/100,000.

INDIAN SCENARIO

According to comparison of incidence rates of all PBCR's(Population Based Cancer Registry) under National Cancer Registry Programme (NCRP India),for the year 2006-2008, Aziwal district of Mizoram showed highest age adjusted incidence rates of 5.6 per lakh population in males, followed by rest of Mizoram state with 3.2/lakh population. In females Imphal west district 2.7 per lakh population registered the highest age adjusted incident rate followed by thiruvanantapurum 2.3 per lakh & Ahmedabad 2.1 per lakh . The overall annual incidence is 3.7/100,000 persons.

ETIOLOGY AND PATHOGENESIS:

CONDITIONS PREDISPOSING TO DEVELOPMENT OF AML:

Environmental factors:

- Radiation[1]
- Benzene[2]
- Alkylating agents

• Topoisomerase II inhibitors

• Tobacco smoke[3]

Acquired diseases:

Clonal myeloid diseases;

• CML

• Primary myelofibrosis

• Essential thrombocythemia

• Polycythemia vera

• PNH

Other hematopoietic disorders:

• Aplastic anemia

• Myeloma

Other disorders:

•HIV

•Thyroid disorders

•Poly endocrine tumours

Inherited or congenital conditions:

•Sibiling with AML

•Rothmund-Thomson syndrome

•Bloom syndrome

•Diamond-Blackfan syndrome

•Down syndrome

•Fanconi syndrome

•Neurofibromatosis

•Noonan syndrome

•Dyskeratosis congenita

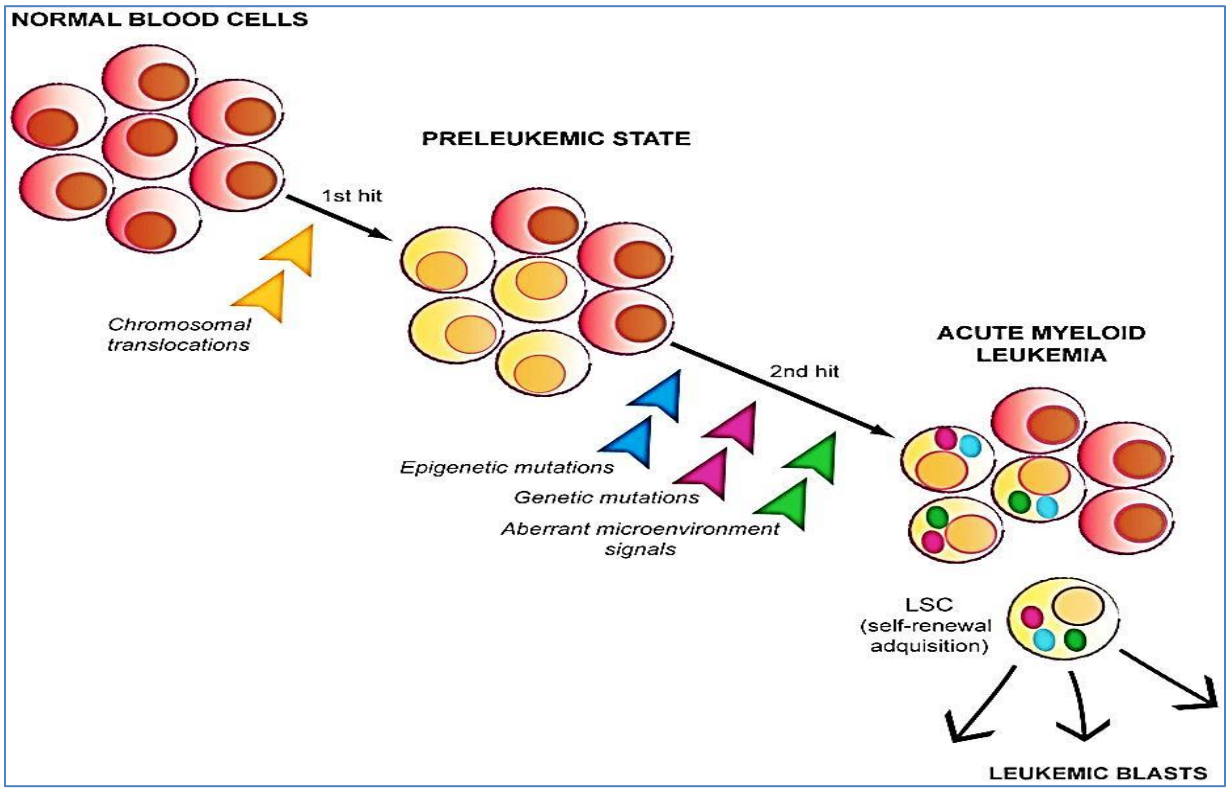
•Ataxia-pancytopenia

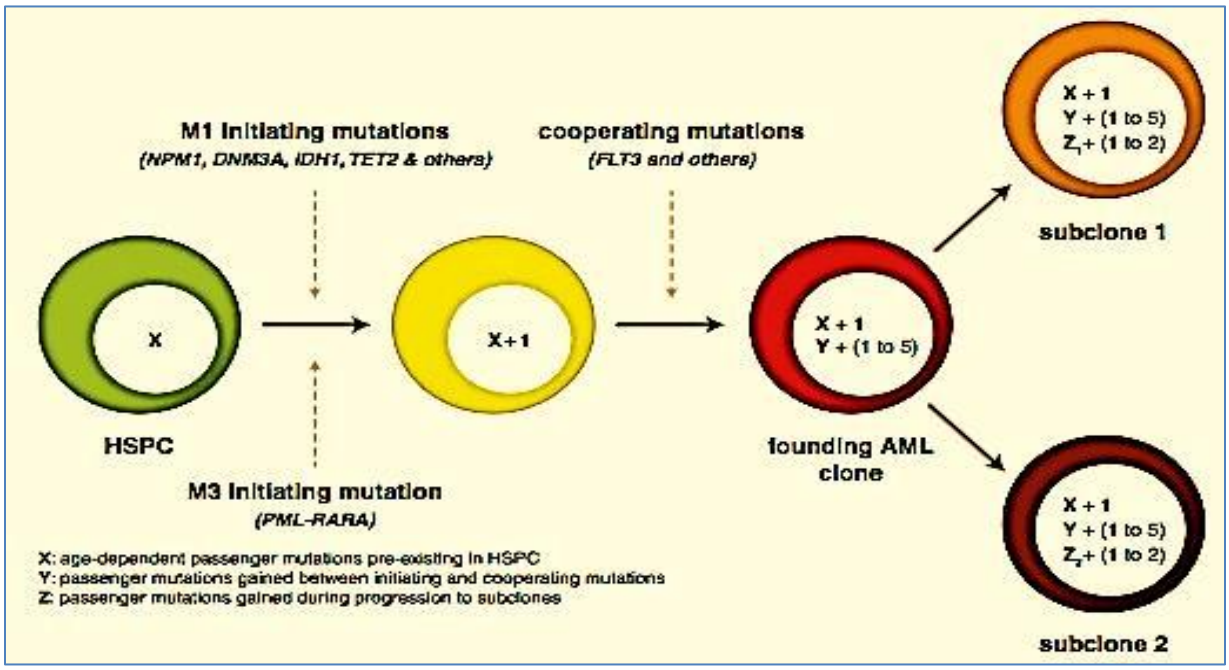
•Familial platelet disaorder

Molecular Pathogenesis:

Hematopoietic multipotential cell or lineage restricted progenitor cell are affected by series of somatic mutations. The acute myeloid leukemia stem cell is found to be CD123 positive ,CD45 dim, CD34 positive , CD38 negative.[4] Chromosomal translocations are the most somatic mutations which results in the fusion gene which produces fusion protein.[5] Fusion protein halts the regulatory sequence controlling the progenitor cells. The cell differentiation , maturation and proliferation are affected by dysregulated signals. In APL the fusion protein prevents the promyelocyte maturation by repressing retinoic acid inducible genes.[6,7] Hence results in leukemia.

AML develpoment as multistage process





Clinical features of acute myeloid leukemia.

The AMLs usually present with some combination of granulocytopenia, anemia, thrombocytopenia, and the appearance of immature cells (blasts) in blood and marrow. The classic presentation of AML in an otherwise healthy patient is most often seen in young adults. Older patients can present with atypical aspects such as a pre-existing myelodysplastic syndrome or a slow onset of the disease (smouldering leukemia). AML in younger patients is a catastrophic illness. The entire clinical history is seldom more than a few weeks long and is characterized by the sudden appearance of fatigue, fever, bacterial infections of the upper respiratory tract, bone pain, and various bleeding manifestations. These are signs and symptoms associated with marked anemia, granulocytopenia, and thrombocytopenia.

Clinical features of anemia are present. Characteristically, symptoms can be out of proportion to the severity of anemia. Thrombocytopenia results in bleeding manifestations. Physical findings in the AML patient include pallor from the anemia, skin and mucous membrane bleeding, aphthous ulcers, gingivitis and pharyngitis, and, on occasion, sternal tenderness. Minor skin infections can occur . Major infections are rare before chemotherapy. Cancer cachyxia and significant

weight loss are common. Palpable hepatospleenomegaly occurs in 30 % of the patients. Lymphadenopathy is seen only in the M4 M5 FAB subtypes of acute myeloid leukemia.

Specific Organ System

Extramedullary infiltration is most common in M4 M5 FAB subtypes of acute myeloid leukemia.[8]

Skin involvement may be

- Leukemia cutis or
- Myeloid sarcoma or
- Nonspecific lesions

There may be involvement of sensory organ like retina ,optic nerve and cochlea.[9]

- ✓ The gastrointestinal symptoms are mostly organic. Patient may present to the dentist for gingival infiltration resulting in tooth extraction. Diarrhoea due to proctitis can occur in M4 and M5 subtypes.
- ✓ Cardiac involvement though frequent but are asymptomatic. Infiltration into the pericardium, myocardium, endocardium and into the conduction system can occur.[10]

- ✓ Kidneys can also be affected .
- ✓ Nervous system involvement is not so common but can occur in monocytic type of acute myeloid leukemia.[11]
- ✓ Bone pain and necrosis can occur.
- ✓ Patients with high levels of primitive blasts (greater than 100,000/μL) can present with a leukostasis syndrome characterized by ischemia of multiple organs and both pulmonary and central nervous system dysfunction (Ball disease). This situation is a true hematologic emergency and needs to be treated immediately with combination chemotherapy and leukopheresis to prevent death.

Chloroma

- ✓ They may present alone without the involvement of bone or marrow. It may be initial manifestation. They are rich in MPO which gives it green colour hence named as chloroma. They can be found anywhere in the body. Patients with chloroma and translocation involving 8 and 21 chromosome have poor prognosis.[11]

LEUKEMIA CLASSIFICATION

Initially it was a purely morphologic classification. However, over the years it has been bolstered by immunohistochemical and immunologic data, using markers for

different stages of myeloid differentiation. Even with this information, the overriding principle of leukemia classification continued to be the placement of the malignant cell in the normal scheme of hematopoietic cell differentiation. The most recent modification of the classification of AML, proposed by a World Health Organization conference, goes a step further. It takes into account both genetic information and whether AML arises de novo or evolves from a myelodysplastic syndrome in the classification . This approach is likely to continue to evolve in the future as new information regarding cell biology, therapeutic response, and outcome becomes available. Gene-expression profiling using microarray technology is expected to have a major impact on leukemia classification

WHO Classification of AML

- AML with recurrent genetic abnormalities
- AML with multilineage dysplasia
- AML and MDS, therapy-related
- AML not otherwise categorized
- AML of ambiguous lineage

FAB classification of acute myeloid leukemias.

Cell Type	*FAB*	*Description*	*Incidence (%)*
Undifferentiated	M1	Blasts with bland characteristics	20
Myeloblastic	M2	Blasts with early granulocytic differentiation	30
Promyelocytic	M3	Clear promyelocytic characteristics	10
Myelomonocytic	M4	A mixture of granulocytic and monocytic characteristics	25
Monocytic	M5	Clear monocytic characteristics	10
Erythroleukemic	M6	Blasts with erythroid characteristics	4
Megakaryocytic	M7	Blasts with megakaryocytic properties	1

Diagnosis

Usually diagnosed by the myeloblasts in the PS . Bone marrow aspiration is mandatorily done for the diagnosis. Bone marrow aspirate is subjected to

- Morphological examination
- Histochemistry
- Immunophenotyping

- Molecular markers study
- Cytogenetic study

COMPLETE BLOOD COUNT

The complete blood count (CBC) will demonstrate varying combinations of anemia, granulocytopenia, and thrombocytopenia, together with the appearance of abnormal immature cells (blasts) in circulation. The total number of blasts will vary from a small percentage of the circulating cells to an overwhelming, uniform population of primitive blasts, exceeding 50,000/μL. Loss of other normal cell elements also helps make the diagnosis. The malignant event responsible for proliferation of the leukemic cell line also blocks normal granulocyte, red blood cell, and platelet production. At the same time, small numbers of normal lymphocytes will still be present. Presence of lymphocytes but absence of normal granulocytes in circulation suggests involvement of the myeloid cell line in the leukemic process. Up to 10% of leukemias can present as aleukemic leukemia, that is, as pancytopenia with few if any blasts in circulation. In this situation, marrow aspirate and biopsy invariably give the diagnosis. In patients who gradually evolve to AML as an endpoint of a myeloproliferative disorder, the pattern of the cytopenia may be quite variable. In addition, some of these patients will demonstrate a macrocytic anemia, marked poikilocytosis with nucleated red blood cells on the peripheral film, or both.

MARROW ASPIRATE AND BIOPSY

A marrow aspirate and biopsy should be obtained in all patients. Sufficient material should be collected for routine morphology and histochemical staining, immunophenotyping, and chromosomal analysis. This process will require several aspirations and may involve several placements of the marrow aspirate needle.A marrow biopsy core should be obtained from a separate site at some distance from the point of marrow aspiration to guarantee an adequate specimen. The biopsy is important to determine overall cellularity, the distribution of the malignant process, and any tendency to fibrosis. Rarely, an AML patient will present with a packed or even necrotic marrow that defies aspiration. In this case, the biopsy is critical.

Morphology

The maturation of myeloid cells in the marrow is relatively easily followed based on morphologic criteria . Several characteristics of the maturation sequence are used in classifying the leukemic cell line. The first of these is the appearance of cytoplasmic granules in the maturing granulocytes. The most primitive (undifferentiated) myeloblasts have no granules and are difficult to distinguish from lymphoblasts. As the cell line matures to the promyelocyte stage, primary granules become abundant and partially obscure the nucleus .Some patients with promyelocytic leukemia have microgranular morphology. Malignant myeloblasts

and promyelocytes can also contain abnormal rod-shaped granules known as Auer rods . When present, Auer rods are by far the best criterion for identifying leukemic myeloid cells in the granulocytic lineage. As myelocytes mature further, they acquire secondary granules that are smaller and more heterogeneous in their staining properties. When cells follow the monocytic differentiation pathway, cytoplasmic granules are never as prominent as those in granulocytes. They are smaller, scantier, and remain pink to purple. The categorization of leukemias as myelomonocytic reflects the difficulty in distinguishing between immature cells of the granulocyte and monocyte lineages. Finally, acute eosinophilic and basophilic (mast cell) leukemias are readily identified from the distinctive granules within the cytoplasm.

These stains are used in conjunction with conventional morphology in the FAB classification system.The second most important morphologic criterion in the detection of a myeloid leukemia is the morphology of the nucleus. The chromatin of immature cells such as myeloblasts is characterized by a very fine, lacy pattern. As the cell matures, the chromatin becomes progressively more coarse or clumped. Moreover, the nature of the clumping is different in granulocytes, lymphocytes, and monocytes. The nucleus in the granulocyte lineage first folds at the metamyelocyte stage and then becomes segmented, whereas the nucleus of the developing monocyte remains indented or horseshoe shaped. Nucleoli are another

sign of immaturity. They are invariably present in immature blasts and are lost during normal maturation. Leukemic cells frequently have a nuclear chromatin pattern that is finer and more immature appearing than that of a corresponding normal cell. They also demonstrate multiple (three to five or more), often large, nucleoli that persist even as the cell cytoplasm matures.

HISTOCHEMISTRY [12]

Histochemical stains help confirm the granule content of a malignant cell line. The peroxidase and specific esterase stains detect the primary granules of myeloid cells. In contrast, the nonspecific esterase stain detects esterase activity in monocytes and is only weakly positive in immature myeloid cells. Both the alkaline phosphatase and periodic acid Schiff (PAS) stains give a strong reaction with mature granulocytes. The most commonly used stains are the following

Peroxidase stain :

This stain detects the myeloperoxidase enzyme contained in the primary granules of myeloid cells. In the presence of hydrogen peroxide, myeloperoxidase releases free oxygen that can then be detected with benzidine or 3-amino-9-ethyl carbazole. The latter reagent gives a reddish-brown reaction product, whereas the benzidine reagent produces a bluish-black product. Myeloperoxidase is abundant in nearly all

mature and immature myeloid cells and is also present in monocytes to a small extent. Recently, it has become possible to detect myeloperoxidase using a monoclonal antibody and flow cytometry. The increased sensitivity of this method has proved very useful in identifying even very immature myeloid leukemias that appear to be peroxidase negative by histochemistry.

Combined esterase stain :

This stain uses two substrates, alpha naphthyl acetate and naphthol ASD chloracetate, to distinguish myelocytes from monocytes. The chloracetate esterase stain (specific esterase) identifies the primary and secondary granules in myeloid cells. It gives a negative or very weak reaction in monocytes. Furthermore, it is resistant to treatment with sodium fluoride. The alpha naphthyl acetate stain (nonspecific esterase) produces a strong reaction in monocytes, which is positive to a varying degree in mature and immature myeloid cells. The monocyte reaction is inhibited by sodium fluoride.

Periodic acid Schiff (PAS) :

This stain involves the oxidation of carbohydrates by periodic acid to aldehyde products. Mature myeloid cells stain intensely red; myeloblasts are usually negative. The Periodic acid Schiff (PAS) stain is useful in separating AML from acute lymphocytic leukemia; lymphoblasts can show heavy blocklike staining.

STAINS	M0	M1	M2	M3	M4	M5	M6	M7
MPO	–	+	+	+	+/-	–	+/-	–
SUDAN BLACK B	–	+	+	+	+/-	–	+/-	–
NSE	–	–	–	–	+	+	–	–
PAS	–	–	–	–	–	+	+	–

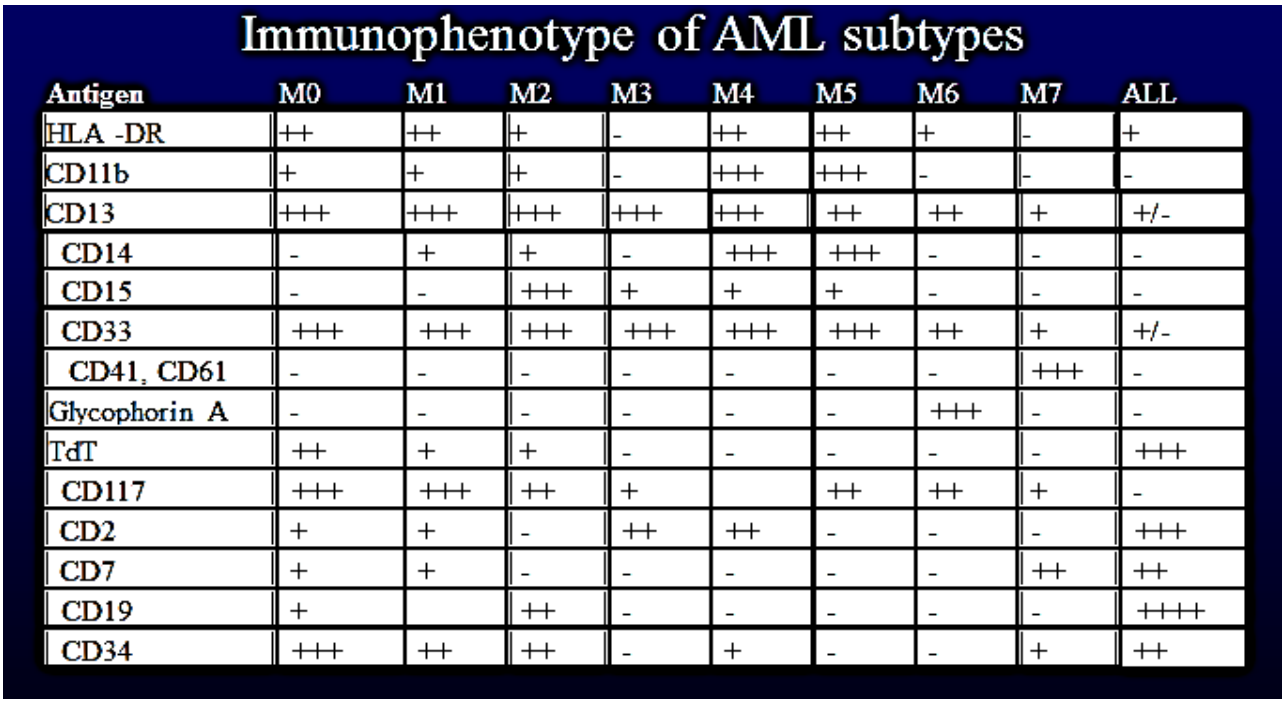
IMMUNOPHENOTYPING

They are most useful in distinguishing myeloid from lymphoid leukemias and in helping determine the lineage of the myeloid leukemias. The immunologic classification of an acute leukemia does not always correlate with the morphologic appearance. For example, cells expressing monocyte markers may or may not have a morphology that suggests monocytic leukemia, and the degree of maturation suggested by the surface markers may not match morphologic features such as cytoplasmic granules and nuclear chromatin. Myeloid leukemias can also show lineage infidelity in that they express markers that are normally not present on the same cell or because they lack markers that should be present on the cells of a given lineage. Finally, it is common to find a heterogeneous expression of markers within the leukemic population. A patient who has nearly 100% blasts by

morphology may show only 50% blasts by marker studies. This condition is usually owing to variations in the intensity of expression of the markers on the cells but could reflect true clonal diversity within the leukemic population.

Immunologic markers on myeloid cells and leukemias.

CD	Other Names	Normal Cells	Utility in AML
13	My7	Mono and myeloid	Distinguish AML from ALL
14	My4	Mature monocytes	Monocytic leukemias
15	My1	Mono and myeloid	Distinguish AML from ALL
33	My9	Mono and myeloid	Most consistent marker in AML
34	My10	Progenitor cells only	Most primitive marker, poor prognosis
41	GPIIb/IIIa	Megakaryocytes	Megakaryocytic leukemia
42	GPIb	Megakaryocytes	Megakaryocytic leukemia
45	HLE	All leukocytes	Frequently decreased in leukemias
117	CKit	Progenitor cells	Expressed with CD34 in most AML
HLA-DR		Mono and myeloid	Nearly always present on AML and ALL
Glycophorin		Erythrocytes	Erythroleukemia



Immunophenotype of AML subtypes

Antigen	M0	M1	M2	M3	M4	M5	M6	M7	ALL
HLA -DR	++	++	+	-	++	++	+	-	+
CD11b	+	+	+	-	+++	+++	-	-	-
CD13	+++	+++	+++	+++	+++	++	++	+	+/-
CD14	-	+	+	-	+++	+++	-	-	-
CD15	-	-	+++	+	+	+	-	-	-
CD33	+++	+++	+++	+++	+++	+++	++	+	+/-
CD41, CD61	-	-	-	-	-	-	-	+++	-
Glycophorin A	-	-	-	-	-	-	+++	-	-
TdT	++	+	+	-	-	-	-	-	+++
CD117	+++	+++	++	+		++	++	+	-
CD2	+	+	-	++	++	-	-	-	+++
CD7	+	+	-	-	-	-	-	++	++
CD19	+		++	-	-	-	-	-	++++
CD34	+++	++	++	-	+	-	-	+	++

Cytogenetics

It helps in deciding the appropriate therapy .It helps in finding the particular subtype of AML. It plays a major role in estimating the prognosis. In 50–60% of cases the cytogenetic evaluation is normal. Both blood and marrow specimens should be collected cytogenetic studies. Chromosomal analysis should be performed using high resolution banding techniques in order to identify common translocations. This type of information is particularly useful in the case of very immature leukemias in which it can be difficult to distinguish between myeloid and lymphoid lineage. The distinctive phenotype and genotype of the malignant cell line can also be used once the patient enters remission to detect relapse and

new mutations at the earliest possible time. The new WHO/FAB classification of the myeloid leukemias recognizes cytogenetic abnormalities as a key factor in the classification of AML. In fact, even in patients with non-diagnostic morphologies and few blasts, the presence of one of these distinctive mutations will make the diagnosis.

RISK STATUS BASED ON VALIDATED CYTOGENETICS ABNORMALITIES[13]

Favourable risk

- ✓ Translocation between the chromosome 8 and 21: FAB M2;
Fused genes are acute myeloid leukaemia 1 protien (*AML1)*and *ETO*.

- ✓ Translocation or inversion in chromosome 16 [14]: FAB M4Eo;
fusion gene *CBF*b/*M*YH11.

- ✓ t(15;17)(q21;q11): FAB M3;
fusion gene PML and RARa.

- ✓ Translocation between the chromosome 11 and 17;
fusion gene PLZF and RARa.

- ✓ Translocation between the chromosome 5 and 17;
fusion gene NPM and RARa.

- ✓ Translocation between the chromosome 11 and 17;
fusion gene NuMA and RARa.

Intermediate risk

- ✓ Normal karyotype: any FAB type

- ✓ + 8: any FAB type
- ✓ abnormal 11q23;fusion gene *MLL*.
- ✓ Others: del(9q); del(7q); +6; +21; +22; –Y and 3–5 complex abnormalities plus other structural or numerical defects not included in the

good risk or poor risk groups.

Poor risk

- ✓ –5/del(5q): any FAB type
- ✓ –7/del(7q): any FAB type
- ✓ Complex karyotypes (>5 abnormalities)

Others: t(6;9)(p23;q34); t(3;3)(q21;q96); 20q; 21q; t(9;22); abn 17p.

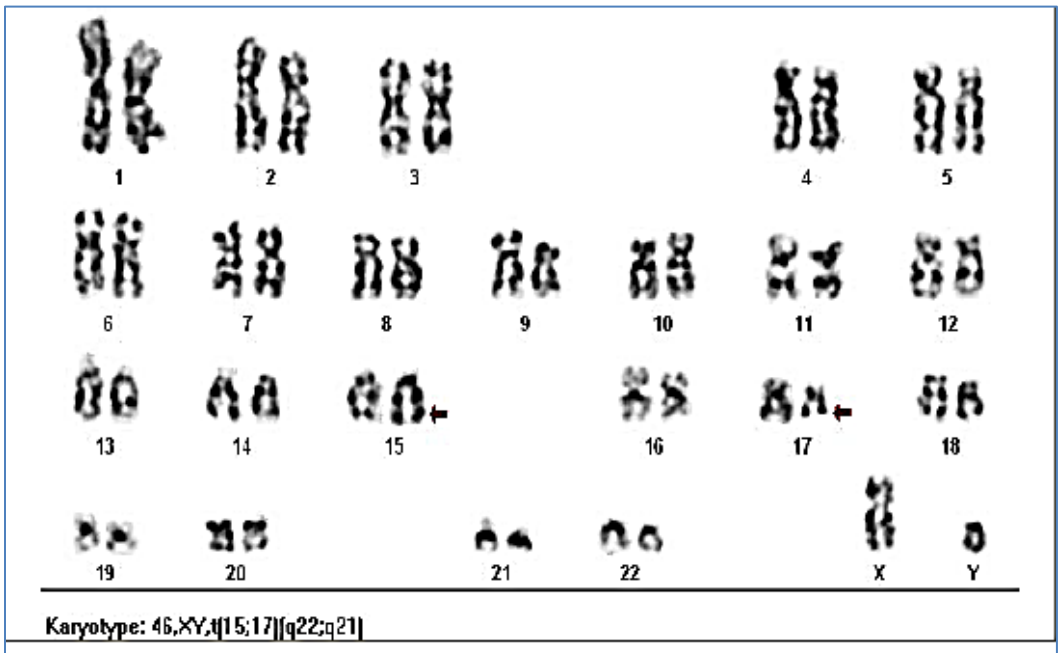


FIGURE 15: Acute Promyelocytic leukemia- – GTG-Banding

Molecular Markers

Molecular markers are more important in the cases having normal cytogenitics.

Many molecular markers with implication on prognosis have been discovered so far. Few common molecular markers with prognostic implictions are FMS-like tyrosine kinase 3 and nucleophosmin mutation .

RISK STATUS BASED ON VALIDATED MOLECULAR ABNORMALITIES

Favourable risk

- ✓ Normal cytogenetics

Nucleophosmin 1 mutation in the absence of ITD mutations in Fms-like tyrosine kinase 3 (FLT-3)[15,16]

OR

Isolated biallelic CEBPA mutation [17-19]

Intermediate risk

- ✓ Translocation between chromosome 8 and 12, inversion or translocation in chromosome 16 with c-KIT mutation

Poor risk

- ✓ Normal cytogenetics
- ✓ ITD mutations in Fms-like tyrosine kinase 3 (FLT-3) [19]

OTHER LABORATORY STUDIES

The growth of the myeloid cell tumor mass is associated with several metabolic abnormalities. The serum lactic dehydrogenase (LDH) level is elevated in most patients without significant changes in other liver chemistries. An elevation mirrors the rate of growth and turnover of the leukemic cells. Another indirect measure of myeloid cell proliferation is the level of vitamin B_{12} binding proteins, specifically transcobalamin III. In patients with myelomonocytic or monocytic leukemia, a high tumor burden is associated with an increased excretion of muramidase (lysozyme) in urine. This process can result in potassium wasting and hypokalemia. Finally, up to 20% of patients may have an elevated serum uric acid level.

AML with recurrent genetic abnormalities

These disorders have genetic abnormalities, the commonest being translocations which create fusion genes that regulate production of abnormal proteins. [20]

Most common are:

- Translocation between the chromosome 8 and 21: They are found in 5-10% of AML cases ,mainly in younger patients. Auer rods are commonly present. Nucleoli are prominent. Blasts express CD13, CD34 and B-cell associated antigen like CD19. Prognosis is better, and this type corresponds to AML-M2 (FAB).[21]

- Translocation or inversion in chromosome16: This corresponds to M4 Eo. Bone marrow shows elements of both granulocytic (including myeloblasts) and monocytic differentiation (monoblasts, promonocytes and monocytes), combined with abnormal eosinophils showing large purple granules along with basophilic granules in a background of eosinophilic granules. WHO classifies it as AML irrespective of blast cell count.[22] In this type blasts are CD13 and CD33 positive and also monocytic markers CD14, CD64 positive. Prognosis is good, they achieve longer complete remissions.[23]
- Translocation between chromosome 15 and 17: Acute promyelocytic leukemia – abnormal promyelocytes – hypergranular or hypogranular are present.

This is equivalent to M3 or M3v in the FAB classification.[24] Numerous Auer rods are present (Faggot cells).This type is usually associated with Disseminated Intravascular Coagulation (DIC). Some cases contain a subset of blasts which are weakly positive for NSE, but MPO is strongly positive. CD 33, CD13, CD117 and CD64 are positive . Patients are sensitive to treatment with All-Trans Retinoic Acid (ATRA) and the prognosis with ATRA therapy is betterthan any other AML cytogenetic subtype.

- Abnormality in q23 of chromosome 11: Frequently seen in children and represents 9% to 12% in them, and 2% of adult AML. This type is positive for CD33, CD65, CD4 and HLA-DR and can be mistaken for M4 or M5 types.

AML with multilineage dysplasia

This is a rare type of AML and demonstrates dysplasia in >50% of cells in 2 or more cell lines including megakaryocytes or a prior history of Myelodysplastic syndrome/Myeloproliferative neoplasm (MDS/MPN). Abnormal erythropoiesis is characterized by ringed sideroblasts, vacuolated cytoplasm and megaloblastic change. Abnormal megakaryocytes are small and have a single lobed nucleus. Granulopoiesis demonstrates lack of granules and bizarre segmented nuclei. This type of AML appears to represent 24-35% of all cases of AML.[25]

Therapy-Related acute myeloid leukemia

This type of AML follows cytotoxic drugs or radiation. MDS occurs first with evidence of marrow failure. Two thirds of cases have multilineage dysplasia and 25% develop refractory anaemia with excess blasts. Usually trilinear dysplasia is seen. One type develops following alkylating agents, another following topoisomerase II inhibitors such doxorubicin therapy.

AML not otherwise categorized

This classification depends on the morphologic and cytochemical characteristics of blasts and their degree of differentiation and includes the following.

• AML, minimally differentiated (M0 in FAB classification) - blasts show no myeloid differentiation and are medium sized, have agranular cytoplasm,

round/oval/indented nucleus with 1-2 nucleoli. Cytochemically, <3% cells are MPO positive and confirmed by CD13, CD33 positivity.

• AML without maturation (M1 in FAB classification) – Blasts>90% of bone marrow non-erythroid cells (NEC). MPO positivity is seen in >3% blasts. Less than ten percentage mature granulocytic or monocytic cells are present in the marrow.

• AML with maturation (M2 in FAB classification) – Blasts form 20-89% of marrow non erythroid cells and may show Auer rods and azurophilic granules. Mature granulocytic cells more than ten percentage of non erythroid cells. Marrow monocytic less than 20% of non erythroid cells. The criteria for acute myelomonocytic leukemia will not be fulfilled.

Acute promyelocytic leukemia – abnormal promyelocytes – hypergranular or hypogranular are present. This is equivalent to M3 or M3v in the FAB classification. Numerous Auer rods are present (Faggot cells).This type is usually associated with Disseminated Intravascular Coagulation (DIC). Some cases contain a subset of blasts which are weakly positive for NSE, but MPO is strongly positive. CD 33, CD13,

• Acute myelomonocytic leukemia (M4 in FAB classification) – Blasts >20% of marrow NEC. Both neutrophilic and monocytic cells and their precursors are present. Each of them more than twenty percentage of the marrow cells . Blasts are MPOpositive. 20-80% of the cells are NSE positive. M4Eo is a distinct entity

with eosinophilic differentiation, demonstrating a specific genetic marker inv 16 and such cases have a good prognosis. The marrow shows promyelocytes, myelocytes with larger eosinophilic/ purple violet granules, along with blasts.

• Acute monoblastic and acute monocytic leukemia (M5a and M5b in FABclassification) –In monoblastic type more than eighty percentage of the cells are monoblasts while in monocytic type more than eighty percentage of the cells are monocytic, mostly monocytes and promonocytes.

• Acute erythroid leukaemia has 2 subtypes-

M6a- Blasts are >20% of the NEC and erythroid cells are >50% of the nucleated cells of the marrow. There is multilineage dysplasia with megaloblastic and dysplastic erythroblasts.[26]

M6b (Pure erythroid leukemia) – erythroblasts constitute more than eighty percentage of marrow cell. Erythroblasts have agranular basophilic cytoplasm. Round nuclei with fine chromatin and 1-2 nucleoli.

• Acute megakaryoblastic leukemia (M7) – In this type, more than fifty percentage of megakaryocytic precursors seen in marrow. The megakaryoblasts are often pleomorphic, having basophilic, often agranular cytoplasm that has pseudopod and bleb formation.Circulating micromegakaryocytes are seen.

• Acute basophilic leukemia- In this type, the primary differentiation is to basophils and the blasts are of medium size with a high nucleus to cytoplasm ratio. Coarsely granular basophilic cytoplasm is seen.

• Acute panmyelosis with myelofibrosis- Acute panmyeloid proliferation with accompanying fibrosis of marrow. They do not meet the criteria for acute myeloid leukemia with myelodysplasia related changes.

Acute Leukaemias of Ambiguous Lineage

These are the cases in which myeloid/ lymphoid origin is not made out .

In less than 4% of cases of leukemia, tests currently available:

(1) cannot determine whether the blasts have a myeloid or lymphoid origin (acute undifferentiated leukemia)

(2) indicate two populations of cells, each having a distinct lineage from myeloid or T or B lymphocytes (acute bilineal leukemia)

(3) indicate that the blasts individually have markers of two or three lines of myeloid, T lymphocytes, and B lymphocytes (acute biphenotypic leukemia). In acute undifferentiated leukemia, the blasts lack any distinguishing characteristics, whereas in the bilineal and biphenotypic forms, the leukemic cells may resemble lymphoblasts, myeloblasts,or monoblasts.

BIPHENOTYPIC LEUKEMIA DIAGNOSIS

It is necessary to score more than 2 points from two separate lineages in order to classify a case as biphenotypic.[26]

Points	*B lineage*	*T lineage*	*Myeloid lineage*
2	CD79a	CD3 (cyt/m)	anti-MPO
	cyt IgM	anti-TCR α/β	(anti-lysozyme)
	cyt CD22	anti-TCR γ/δ	
1	CD19	CD2	CD13
	CD10	CD5	CD33
	CD20	CD8	CDw65
		CD10	CD117
0.5	TdT	TdT	CD14
	CD24	CD7	CD15
		CD1a	CD64

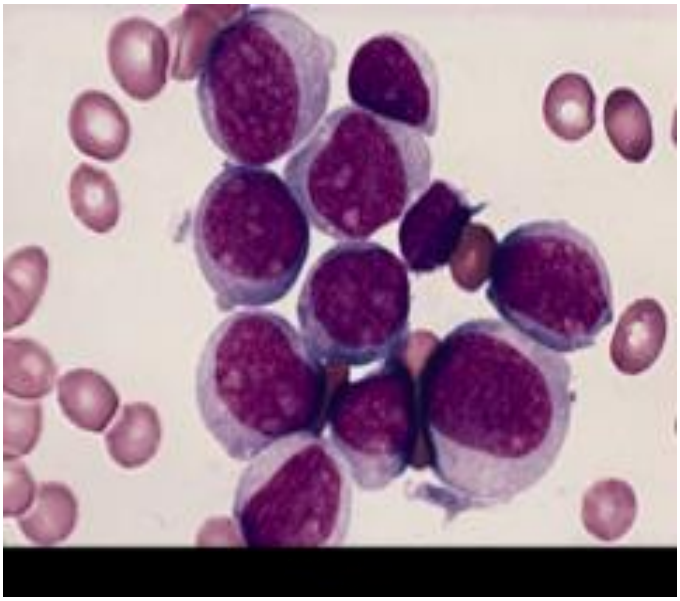
Special cytologic , clinical,and laboaratory features of subtypes of AML

Acute myeloblastic leukemia (M0,M1,M2)

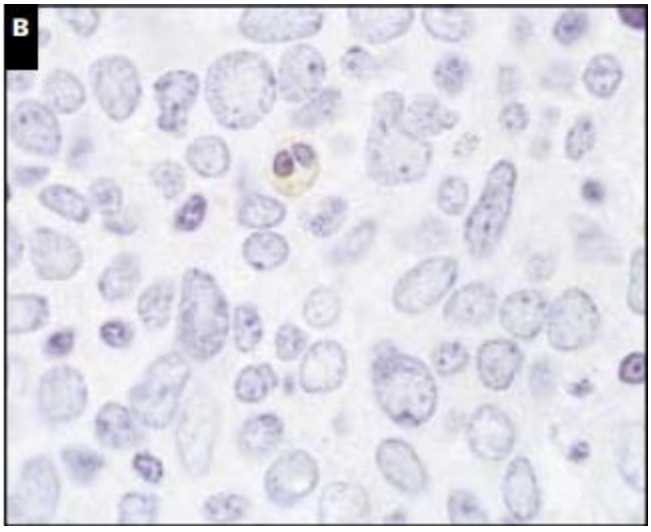
Cytologic Features

1. 20 to 90% blasts cells are seen. Distinct nucleolus and fine reticular nucleus are seen. Auer bodies are rare.
2. Blasts are positive for MPO , chloroacetate esterase and negative for NSE.

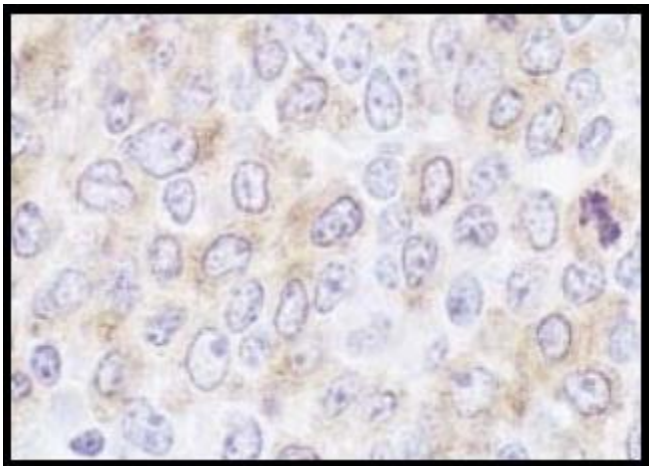
AML M0



MYELOPEROXIDASE NEGATIVE STAINING AML M0



AML M1 MYELOPEROXIDASE POSITIVE STAINING



Special Clinical Features

1. Most common in adults and infants.

2. Three subtypes are included in acute myeloblastic leukaemia.
3.

Special Laboratory Features

1. Acute myeloid leukaemia protein 1 and FLT3/ITD mutations occur in about 20–25% of cases.

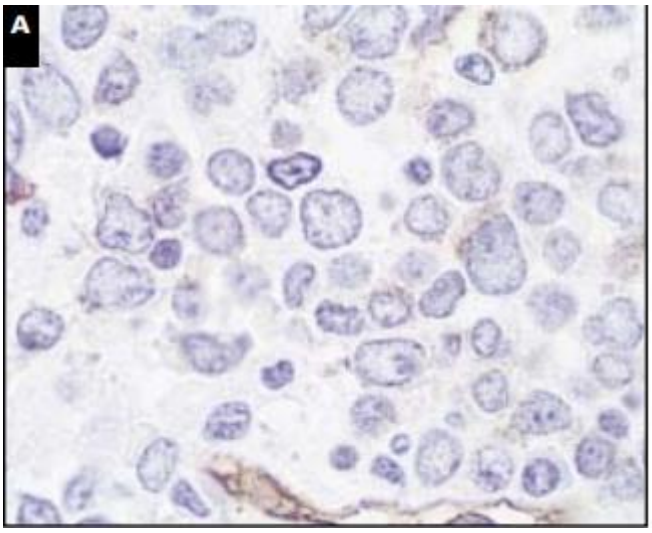
2. M0 subtype is CD34 CD13 or CD33positive.

3. CD13 and CD33 are expressed in M1.Positive for myeloperoxidase by cytochemistry.

4. M2 AML is associated with translocation of chromosome 8 and 21.

5. Complex cytogenetic abnormalities are common.

AML M0 Positive immunostaining CD34



Acute promyelocytic leukemia (M3)

Cytologic Features

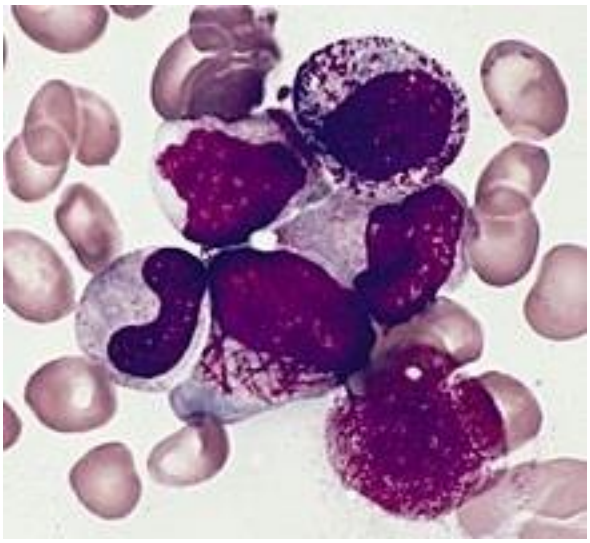
1.Abnormal promyelocytes are present. Nucleus is kidney shaped .

2.Hypergranular acute promyelocytic leukemic cells are called faggot cells. A variant of M3 has microgranules. [27-28]

3.Intensely positive for MPO.

4. Branched auer rods can be seen.

A faggot cell (bottom left)



Special Clinical Features

1. Usually in adults.

2. Hypofibrinogenemia and hemorrhage are common.[30]

3. Blasts mature in response to ATRA.

Special Laboratory Features

1.Translocation between chromosome 15 and 17 seen.

2. Cells are HLA-DR negative.

Acute myelomonocytic leukemia (M4, M4Eo)

Cytologic Features

1.Blast population consist of both myelobasts and monoblasts.

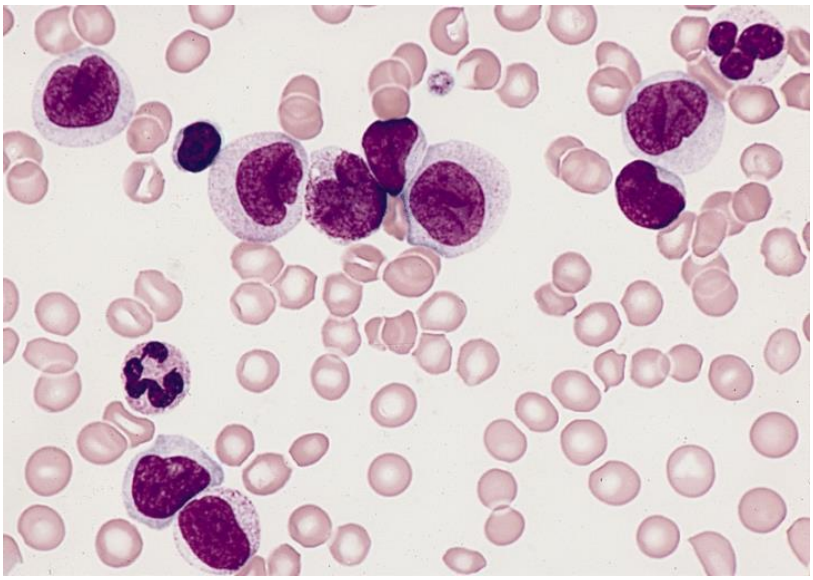
2.MPO, Sudan Black B, chloroacetate esterase, and NSE positive cells.

3. M4Eo variant has marrow eosinophilia.

GUM INFILTERATION

AML-M4

MYELOBLAST AND MONOBLASTS SEEN



Special Clinical Features

1 Extramedullary infiltration is common.[31]

2. Mildly elevated serum and urine lysozyme.

Special Laboratory Features

1. inversion or translocation of chromosome 16 is associated with eosinophilic variant.

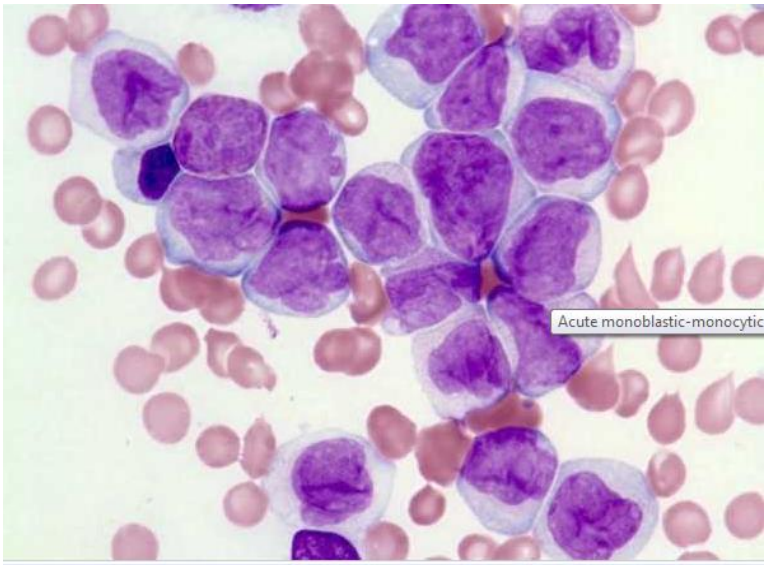
Acute monocytic leukemia (M5)

Cytologic Features

1. Large blasts with low N:C ratio lower than myeloblast with fine granular cytoplasm.

2. NSP +ve inhibited by NaF

3. Auer rods are rare



[ACUTE MONOCYTIC LEUKEMIA (AML-M5b)]. Another example of acute monocytic leukemia with numerous promonocytes is shown. Histochemical staining shows positivity for non-specific esterase and alpha-butyryl esterase (NBE).

Special Clinical Features

1. Seen in children or young adults.

2. Extramedullary involement is commonly seen.

3. Disseminated intravascular coagulation can occur.

4.Lymphadenopathy, gum infiltration and central nervous system infiltration can occur.

Special Laboratory Features

1Translocation of chromosome 4 and 11 are common in infants.

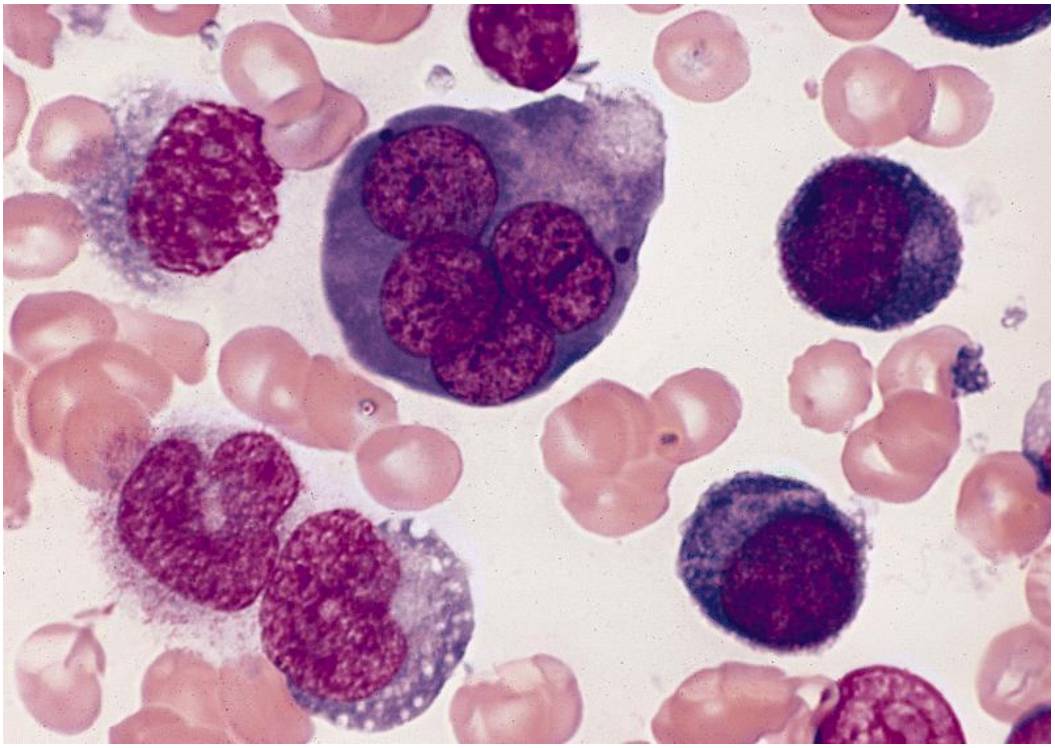
2.Rearrangement of q11;q23 very frequent.

Acute erythroid leukemia (M6)

Cytologic Features

1.Abnormal erythroid cells are seen.[32]

AML M6- Multinucleated erythroblast has megaloblastoid chromatin



Special Clinical Features

1. Pancytopenia common at diagnosis.

Special Laboratory Features

1. Cells reactive with antihemoglobin antibody.[33]

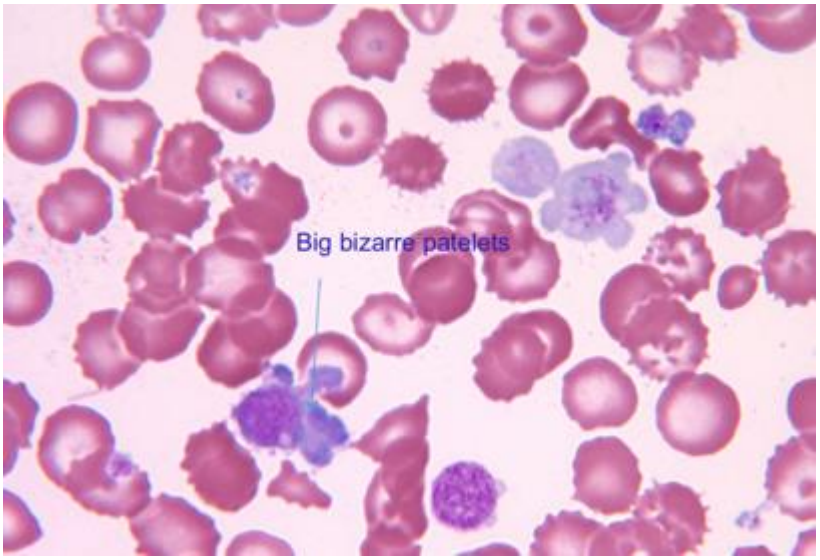
Acute megakaryocytic leukemia (M7)

Cytologic Features

1.Bleb forming blasts are seen.

2. Megakaryoblasts are seen with dense ,homogenous chromatin and irregular cytoplasmic border.

AML M7 – BIG BIZARRE PLATELETS



Special Clinical Features

1. Usually presents with pancytopenia.

2. Markedly elevated serum lactic dehydrogenase levels.

3.Intense myelofibrosis results in dry tap .[34]

4. Associated with Down syndrome.

Special Laboratory Features

1. Antigens of VWF, and gp Ib , IIb/IIIa , IIIa on blast cells.

2. Platelet peroxidase positive.

Acute eosinophilic leukaemia

Cytologic Features

1. Cells containing eosinophilic granules and blasts are seen.

Special Clinical Features

1. Hepatospleenomegaly and lymphadenopathy are present.[35]

Special Laboratory Features

1. Cyanide-resistant peroxidase stains eosinophilic granules.[36]

2. Charcot Leyden crystals can be found in infiltrated sites.

Acute basophilic leukaemia

Cytologic Features

1.Cells with basophilic granules mixed with blasts are seen.

Special Clinical Features

1. Hepatospleenomegaly present.

2. Urticarial rash, headaches, GIT symptoms are present.

Special Laboratory Features

1. CD11b+ve, CD9+ve,CD123+ve,CD25+ve.[37]

2.Cells are +ve for toluidine blue.

3.Histamines in urine and blood are high.

Acute mast cell leukaemia

Cytologic Features

1. Granular mast cells are seen.

Special Clinical Features

1.Patient may present with the complaints of flushing of face, fever ,pruritis,and headache.

2.Peptic ulcer, bone pain, diarrhea are common .

3.Hepatospleenomegaly is commonly present.

4.Hemorrhagic manifestations may be present.

Special Laboratory Features

1. CD117+ve,CD13+ve, CD68+ve, CD33+ve are positive.[38]

2. Hyperhistaminemia and hyperhistaminuria.

3. Positive staining for tryptase

4.Serum tryptase elevated.

An important element in diagnosing AML is the speed of the workup. This is especially true when AML presents as a precipitous illness in an otherwise healthy young adult, because successful management depends on the early initiation of appropriate chemotherapy. The time element is less important in patients who gradually evolve from a myeloproliferative or myelodysplastic disease into AML. In this situation, it can be to the patient's advantage to delay therapy until it is absolutely necessary.

It is very important to determine whether the AML is secondary to some other factor such as previous chemotherapy or radiation exposure. AML is the most common form of secondary malignancy following previous tumor therapy. In addition, the incidence of AML as the final outcome in patients with myeloproliferative/dysplastic diseases, such as RAEB (refractory anemia with

excess blasts), polycythemia vera, and myelofibrosis may be increased by treatment with specific chemotherapeutic agents. Both aplastic anemia and AML have also been associated with benzene exposure. These relationships are important since secondary AML generally carries a worse prognosis. The patient may be slow to achieve remission, relapse rapidly, or fail to recover normal hematopoiesis following chemotherapy.

Because of the need for a rapid workup and treatment decision, the initial classification of a myeloid leukemia is most often based on cellular morphology and histochemical staining, using the old FAB system . The key decision points are the following:

- Does the patient have acute myelocytic versus acute lymphocytic leukemia?
- To which FAB category does the patient belong?

Therapy can be and should be initiated based on this information. It is unnecessary and unwise to wait for the results from immunophenotyping and chromosomal analysis. Although these results may affect long-term management and prognosis, they are not important in guiding the initial treatment decision.

The patient should also be routinely evaluated for a coagulopathy. This process is very important in patients with acute promyelocytic leukemia (M3). Many of these patients present with ongoing DIC and a severe bleeding tendency, which can

worsen in the early phases of treatment. A full coagulation profile including a platelet count, PT, PTT, thrombin time, fibrinogen level, split product or D-dimer level (or both) should be measured prior to initiating chemotherapy.

The final classification of the patient's leukemia will reflect the studies of morphology, immunophenotype, and cytogenetics, and the relationship to preexi**sting preleukemic states .**

PROGNOSTIC FACTORS IN AML

FACTORS	**Factor Favorable**	**Unfavorable**
Age	<45 y, <2 y	>60 y
ECOG PS	0–1	>1
Leukemia	*De novo*	Antecedent hematologic disorder
Infection	Absent	Present
Prior chemotherapy	No	Yes
Leukocytosis	<30,000/mm^3	>50,000/mm^3
Platelet count	>30000/mm^3	<30000/mm^3
Serum LDH	Normal	Elevated
Extramedullary disease	Absent	Present
CNS disease	Absent	Present

Cytoreduction	Rapid	Delayed
FACTORS	**Factor Favorable**	**Unfavorable**
Eosinophils	Present	Absent
Megaloblastic erythroids	Absent	Present
Dysplastic megakaryocytes	Absent	Present
FAB subtypes	M2, M3, M4	M0, M6, M7
Blast %	< 5% blasts on day 14 marrow predicts for complete remission	>70 %

Treatment of AML (except M3)

General Guidelines

Reliable vascular access is essential and usually requires placement of an in-dwelling catheter The patient should be hydrated and receive allopurinol 300 to 600 mg/day by mouth to prevent hyperuricemia as tumor cells are lysed. Serum electrolytes and CBCs need to be monitored on a daily or every-other-day basis. In those patients who are at risk for DIC, a full coagulation profile should be

measured daily for the first several days of chemotherapy. Hypofibrinogenemia (fibrinogen less than 100 mg/dL) should be corrected by infusion of cryoprecipitate, whereas marked prolongations of the PT and PTT may be corrected with fresh frozen plasma. Promyelocytic leukemia patients may benefit from being prophylactically anticoagulated with heparin using a moderate-dose protocol . If bleeding is life-threatening, the patient should be treated with platelet transfusions and infusions of cryoprecipitate to correct hypofibrinogenemia (fibrinogen levels less than 100 mg/dL).In the absence of severe cardiovascular disease, the hematocrit should remain in the vicinity of 25 to 30% to decrease the frequency of red blood cell transfusions. Platelet transfusions should only be given when the platelet count falls below 10,000/μL in the patient with little or no bleeding, or 20,000/μL if the patient has significant mucous membrane bleeding or tends to bleed from venipuncture sites.

The treatment of ongoing infection must be very aggressive, and potential sources of infection should be eliminated. For example, infected teeth should be pulled, abscesses drained, and foreign bodies such as infected intravenous catheters removed and replaced. Good nursing care of the mouth, skin, and rectum is very important. Oral prophylaxis to prevent infection using an oral fluoroquinolone such as ciprofloxacin can be used. It is well tolerated and preserves the anaerobic gastrointestinal flora, thereby avoiding the problem of fungal overgrowth in the

intestinal tract. It also appears to be effective in reducing the frequency of systemic infections with gram-negative bacteria.

Prophylactic antifungal therapy with nystatin, miconazole, and ketoconazole has been used in the past to prevent yeast colonization of the gastrointestinal tract as a source for systemic candidiasis. Newer antifungals, including fluconazole, may be more effective. Empiric antiviral therapy has also been recommended.

Empiric Antibiotic Therapy

If patients are not already febrile at the time of diagnosis, they soon become febrile with treatment. They should have thorough cultures to identify specific organisms. But even before a specific site of infection is identified, they need to receive empiric antibiotic therapy. The choice of antibiotics will depend to some extent on what organisms are seen in each institution. The usual approach is to provide broad-spectrum coverage for both gram-negative and gram-positive organisms. A popular combination of antibiotics for this purpose is an aminoglycoside (gentamicin, tobramycin, or amikacin) together with an antipseudomonal penicillin (carbenicillin, ticarcillin, mezlocillin, or piperacillin).

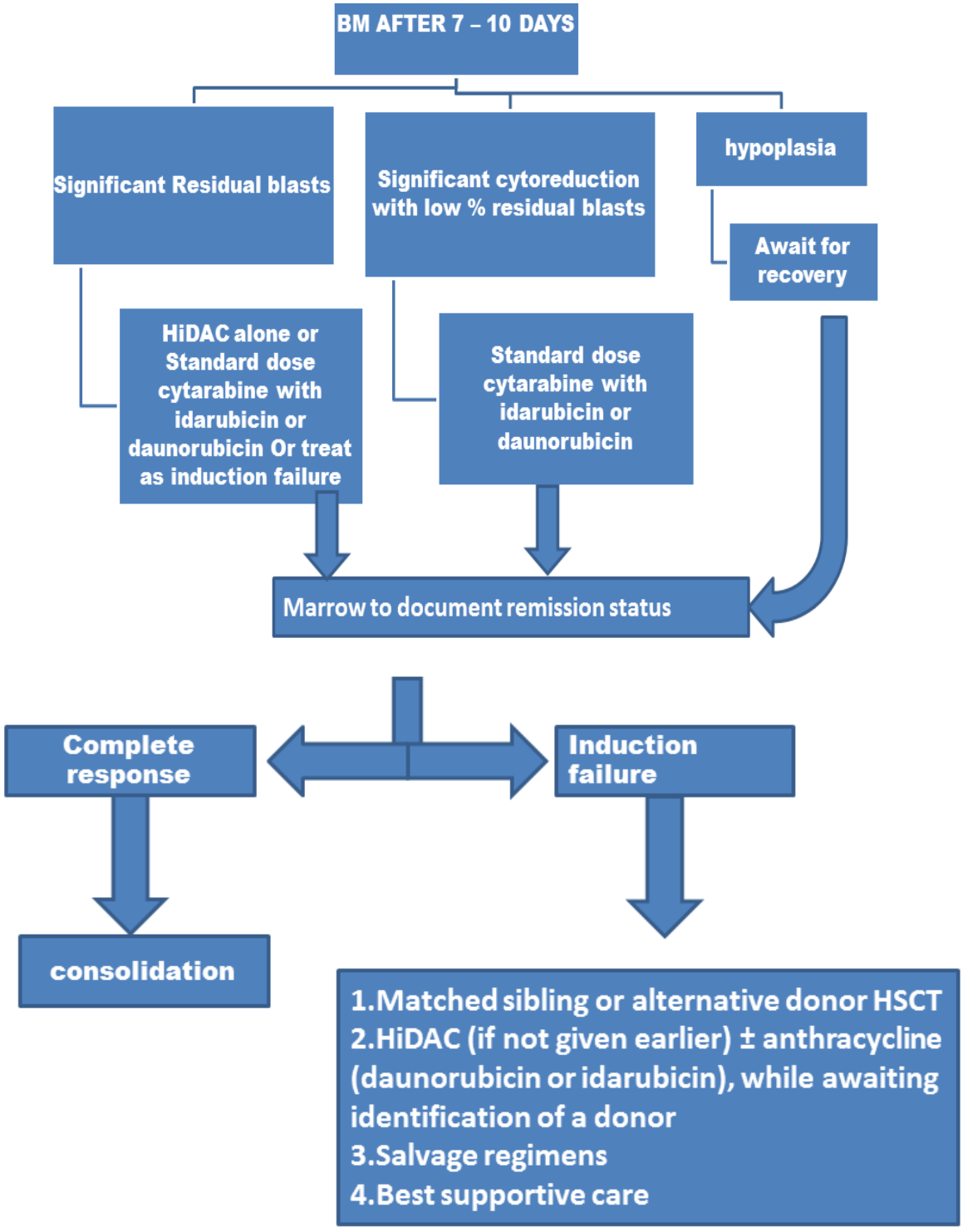
Induction :Age <60 y

- ✓ Cytarabine(standard dose) 100-200 mg per m^2 for 7 d plus idarubicin 12 mg per m^2 or daunorubicin 90 mg per m^2 for 3 days (category 1)[39].
- ✓ Cytarabine(standard dose) plus daunorubicin 60 mg per m^2 for 3 d and cladribine 5 mg per m^2 for 5 d (category 1).
- ✓ Cytarabine (High dose) 2 g per m^2 every 12 hours for 6 d or 3 g per m^2 every 12 hours for 4 d plus idarubicin 12 mg per m^2 or daunorubicin 60 mg perm^2 for 3 d (1 cycle) (category 2B).
- ✓ Predictable complications such as anemia, thrombocytopenia, a coagulopathy, or infection should never delay therapy since they are unlikely to improve until a remission is obtained. They should be treated with appropriate transfusions and antibiotics at the same time the chemotherapy is begun. The goal of therapy is to ablate the leukemic cell line and allow normal progenitor cells to repopulate the marrow. This process will require a period of marrow aplasia and marked granulocytopenia that can extend for 1 to 4 weeks or even longer.

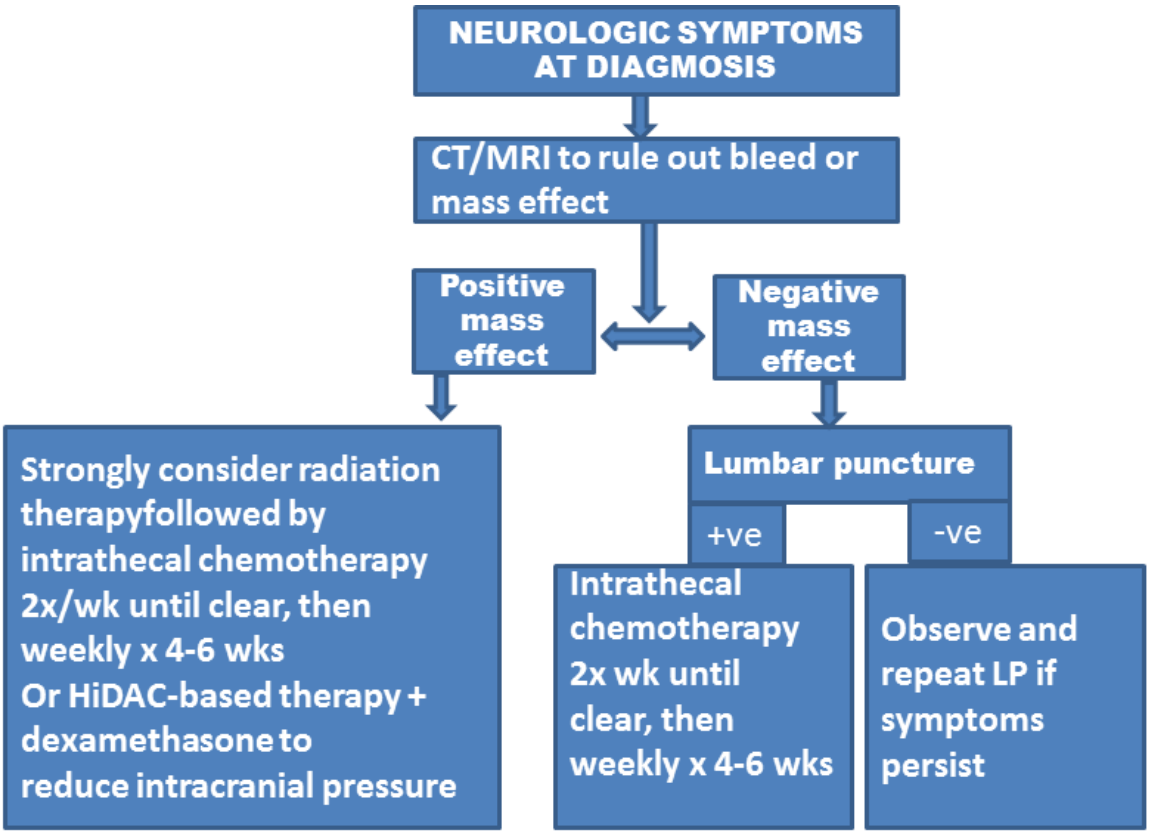
RISKSTATUS (cytogenetics and molecular abnormalities)	**POST REMISSION THERAPY**
Better risk	Cytarabine (high dose) 3-4 cycles (category 1)/ or cytarabine(high dose) 1-2 cycles followed by autologous *Hematopoietic stem cell transplantation* (category 2B)
Intermediate risk	*Hematopoietic stem cell transplantation* or cytarabine(high dose) x 3-4 cycles
Poor risk	Matched sibling or alternative donor HSCT

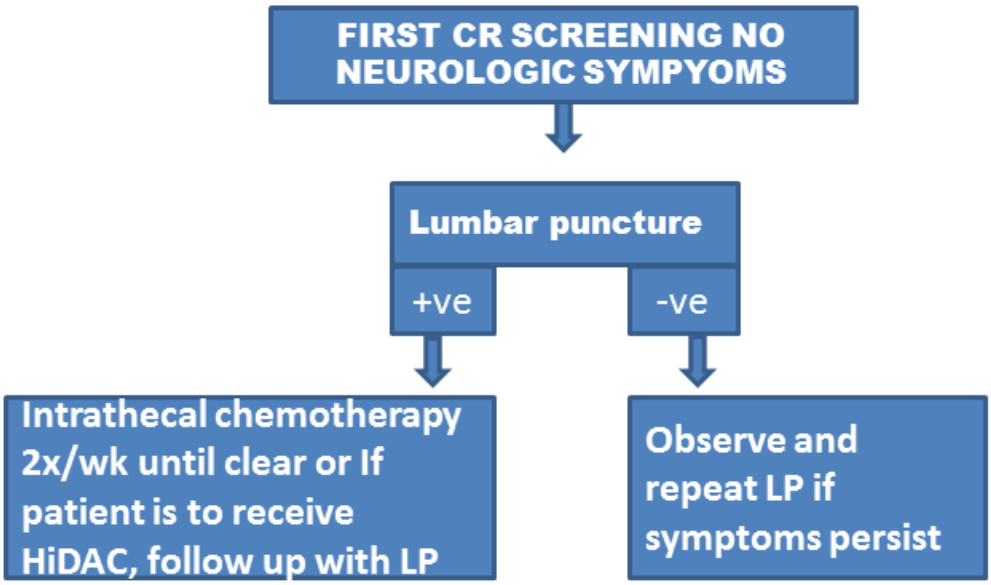
A careful examination of the patient, repeated chest x-ray, and blood cultures should be obtained with each episode of chills and fever, and the clinician should look for a drug-resistant bacterium or fungus.

POST INDUCTION THERAPY



EVALUATION AND TREATMENT OF CNS LEUKEMIA





AGE>60 yrs

Low-intensity therapy may be more appropriate for elderly patients or relatively unfit patients with comorbidities (subcutaneous cytarabine, 5-azacytidine, decitabine). Based on the ECOG performance status low intensity therapy or standard chemotherapy is chosen.

Treatment of APL (M3)

Induction

All trans retinoic acid is used . It is given with daunorubicin or idarubicin. Another option is ATRA + arsenic trioxide.[40] Patient who can not tolerate cytotoxic therapy can be given the above regimen. Arsenic trioxide dose given is 0.15 mg/kg/day IV till remission occurs plus ATRA 45 mg/m^2/day in 2 divided doses.[41]

Consolidation

Post-remission treatment drugs options include:

- ✓ Anthracycline + All-trans-retinoic acid for a few cycles.
- ✓ Anthracycline + cytarabine for at least 2 cycles .
- ✓ Arsenic trioxide for 2 cycles followed by All-trans-retinoic acid + an anthracycline for 2 cycles .
- ✓ All-trans-retinoic acid + Arsenic trioxide for several cycles

Maintenance

ATRA for at least one year. Sometimes low doses of the chemotherapy drugs 6-mercaptopurine and methotrexate are given as well.[42]

ACUTE MYELOID LEUKEMIA RESPONSE CRITERIA [43-45]

Morphologic leukemia free state

- ✓ Bone marrow aspirate containing less than five percentage of myeloblast .
- ✓ No extramedullary disease.
- ✓ Absence of Auer rods .

Complete remission

Morphologic CR

- ✓ PMN >1000/mm^3
- ✓ Platelets count >100,000/mm^3
- ✓ No residual evidence of extramedullary disease
- ✓ Patient independent of transfusions

Cytogenetic complete response

- ✓ Previously abnormal cytogenetics becomes normal.

Molecular complete response-

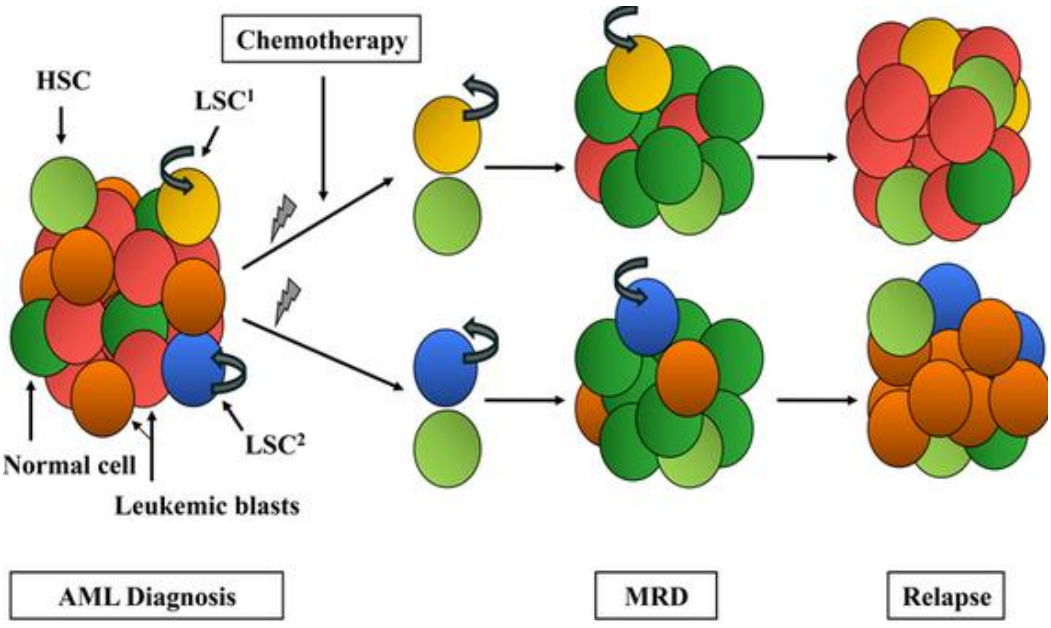
- ✓ RT-PCR negative

Partial remission

- ✓ There is a 50% reduction in the % of blasts to 5% - 25% in the BMA.
- ✓ Blood count is normal.

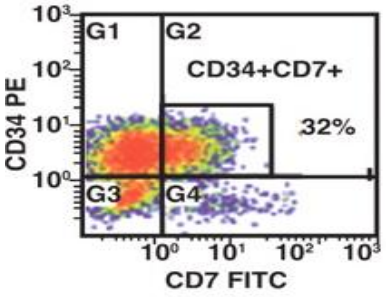
Detection of minimal residual disease

Few leukemic cells which left after treatment which could not be detected by routine bone marrow examination is called minimal residual disease. PCR test and multiparameter flowcytometry are considered the gold standard for MRD monitoring in AML..

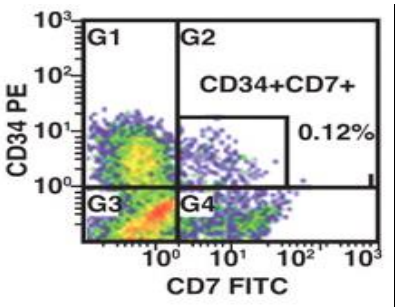


HSC, normal hematopoietic stem cell, LSC, leukemic stem cell

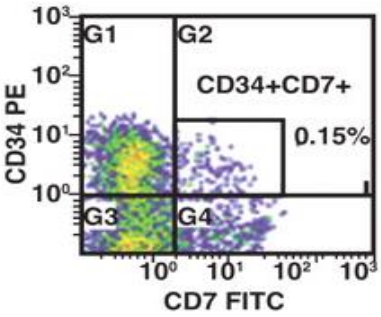
Detection of MRD in consecutive samples from a patient in still remisssion



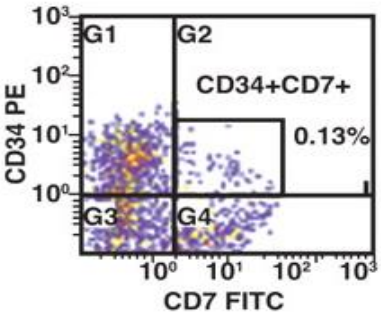
BM: AML at Diagnosis



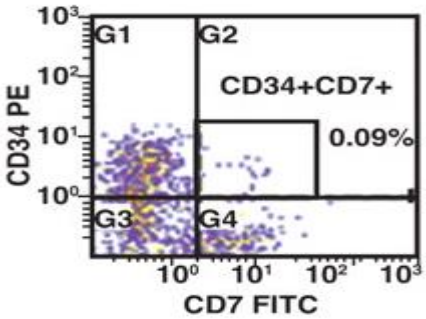
After Induction



After Consolidation I



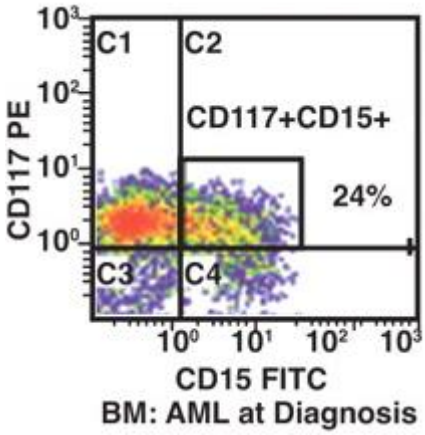
After Consolidation II

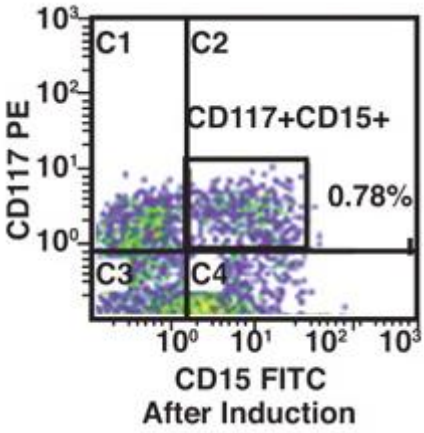


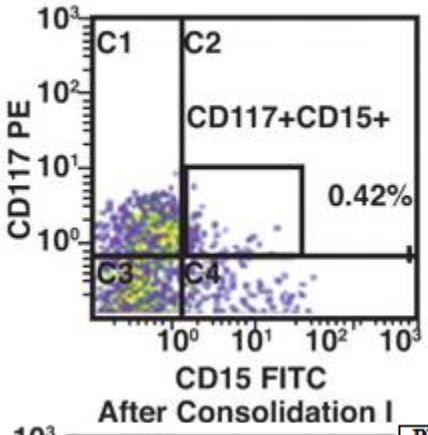
Follow-up

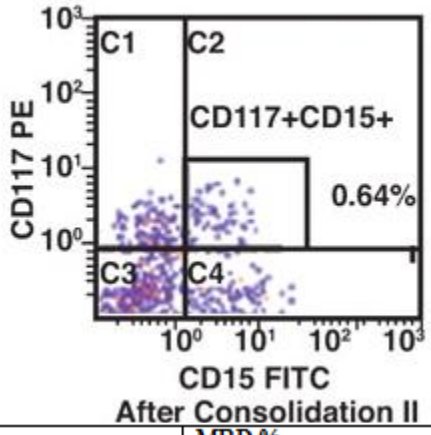
PHASE	MRD %
AFTER INDUCTION	0.12
CONSOLIDATIONI	0.15
CONSOLIDATIONII	0.13
FOLLOW UP	0.09

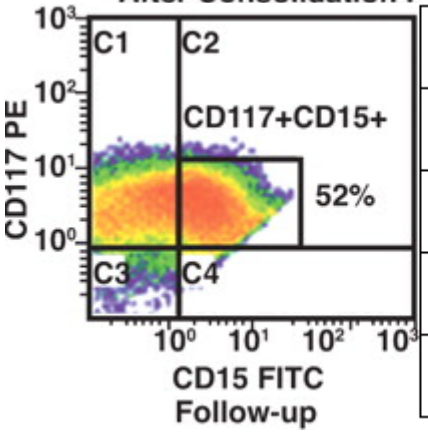
Detection of MRD in consecutive samples from a patient with relapse











PHASE	MRD %
AFTER INDUCTION	0.78
CONSOLIDATIONI	0.42
CONSOLIDATIONII	0.64
FOLLOW UP	52

Sequential monitoring MRD can accurately predict relapse, thus opening the way to risk-directed therapy . MRD can be used to guide the risk adapted treatment.[46] MRD-negative remission leads to prolonged duration of CR. Thus achieving MRD-negative remission represents a gold standard. [47] Recently, a trial of prospective MRD-driven therapy has shown improvement of outcome in high-risk patients.[48] Hence intensified therapy can be given to the patients with MRD during clinical remission.[49]

Aberrant phenotype in AML

Types:

- ✓ Co-expressed lymphoid lineage markers.
- ✓ Asynchronous antigen expression : Mature antigens are co-expressed with immature antigens.
- ✓ Overexpressed antigens[50]

Proposed classification of the immunological markers

Markers	B-lymphoid	T-lymphoid	Myeloid
Lineage-specific markers	IgM (cyt) CD22 (m/cyt)	CD3 (m/cyt) anti-TCR	MPO/NSE (>3%)
Lineage-associated markers	TdT	TdT	CD117
	CytCD79a	CD1a	CD13
	CD 19	CD2	CD14
	CD20	CD5	CD15
	CD 10	CD7	CD16
		CD4/CD8	CD33
		CD10	CD64

Flowcytometry

It is a technology which measure and analyze multiple physical parameter of cells. Relative fluorescence intensity, granularity ,size and internal complexity are the physicical characteristics of the cell which are measured and analyzed. The cells when flowing in a fluid stream cause the scattering of the laser light. These characteristics are coupled to electronic coupling system.

It has three main systems:

• The fluidics system:

Fluid stream in which the cells flow .

• The optics system:

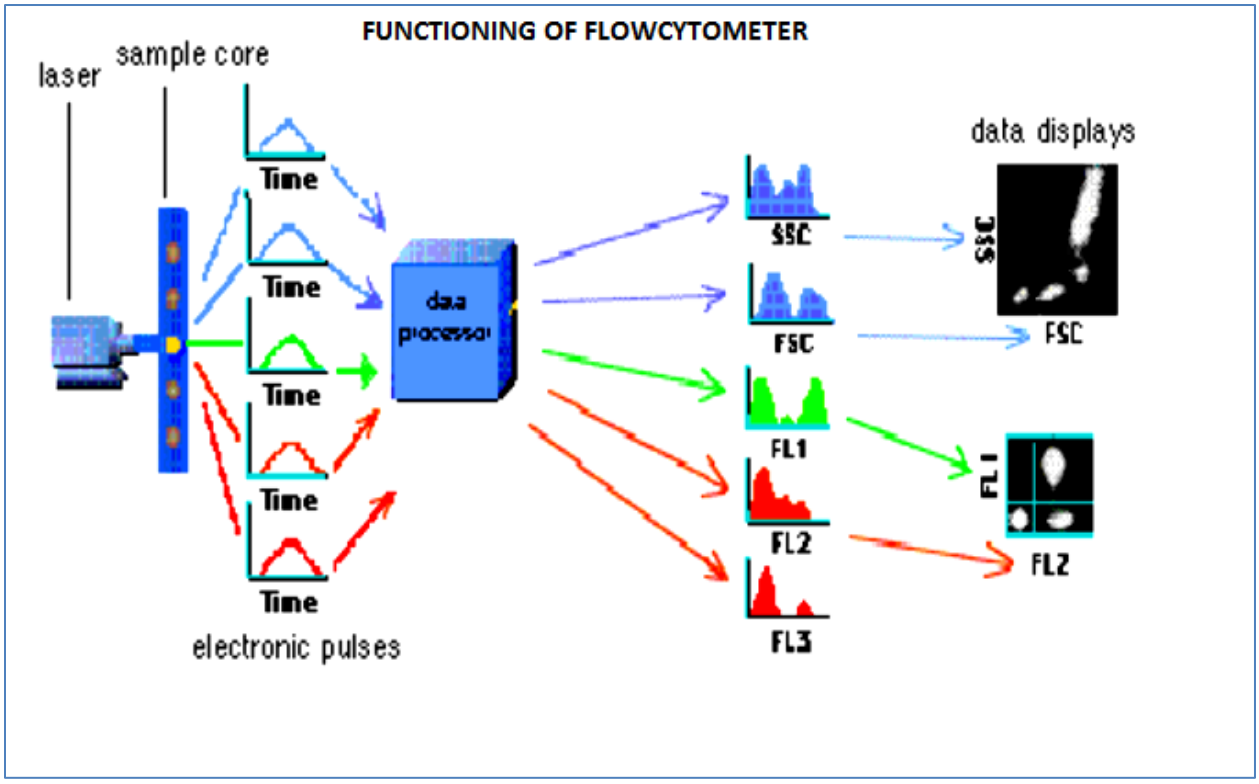
The optical filters and laser beam which illuminates the cells.

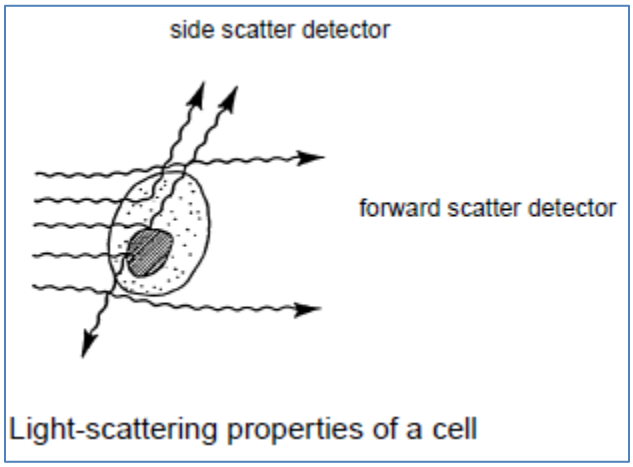
• The electronics system :

Electronic system converts the light signals are into electronic signals which can be processed by the computer.

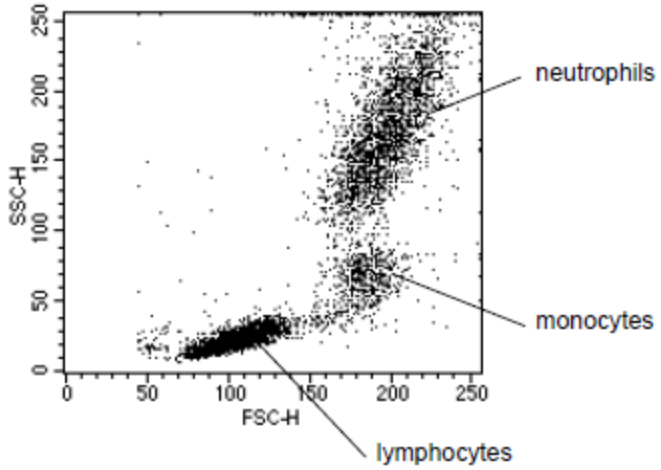
Light Scatter

There are two types of light scatter: forward scattered light (FSC)and side scattered light(SSC)


FUNCTIONING OF FLOWCYTOMETER
laser
sample core
Time
Time
Time
Time
Time
data processor
electronic pulses
SSC
FSC
FL1
FL2
FL3
data displays
SSC
FSC
FL1
FL2


side scatter detector
forward scatter detector
Light-scattering properties of a cell

Correlated measurements of FSC and SSC can allow for differentiation of cell types in a heterogeneous cell population.



Cell subpopulations based on FSC vs SSC

Figure 3-2

Fluorescent dye conjugated with a monoclonal antibody against the individual antigenic surface markers of the cell can be used to identify blast population .

Gating

Graphical boundary can be made to define the blast population by gating.

Differentiation of cell size can be made out by FSC/SSC gating.

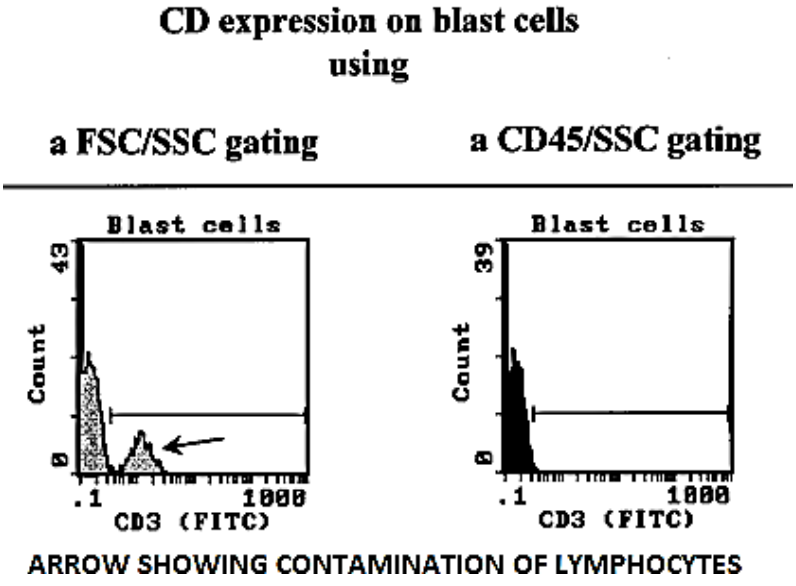
CD45/side scatter (SSC) gating

Precursor cells and blasts have low expression of CD45.CD45 is highly expressed by lymphocytes and monocytes. Hence the blast population can be easily identified. The conventional Forward scatter /SSC gating miscalculates the leukemic cell % . Thus CD45/side scatter (SSC) gating

(1) Clearly distinguishes leukemic blast cells from normal cells.

(2) Exclusion of immunophenotyping of normal cells.

(3) Identification of heterogeneous blasts.



Morphological study of leukemic cells with cytohistochemistry and cytogenitics study can diagnose leukemias. Few cases cannot be diagnosed by the above methods. Such cases can be diagnosed by immunohistochemistry by flowcytometry**.** Analysis of multiple special characteristics of single cells can be done rapidly. The information obtained is both qualitative and quantitative**.**

One of the most important use of flowcytometry is immunophenotyping. Acute leukemias can be accurately diagnosed and classified. Prognostic associations can also be made out

Association between the genetic abnormalities in AML and their immunophenotype

- **AML with t (8;21):**

✓ **Khoury et al, 2003**

They are strongly positive for MPO, HLADR, CD34, and CD13 . CD33is weekly expressed. CD19, cCD79a and CD56 are frequently observed. CD56 expression is associated with poor prognosis in AML.[51]

✓ **Chen SW et al ,2008**

Co-expression of CD19 and CD56 is found in higher frequency in AML with translocation between chromosome 15 and 17.[52]

✓ **AML with translocation or inversion in chromosome 16:**

Dunphy, 1999; Medeiros et al, 2010

CD2 is the most common lymphoid associated antigens expressed. Asynchronous antigen expression is the most common type of aberrant phenotype found.[53]

- **APL with t(15;17):**

✓ **Paietta et al, 2004**

HLA-DR and CD34 are poorly expressed. There is a strong expression of CD117. 20 % of the patient express CD56 . CD65 and CD 15 ,which are granulocytic markers are not expressed.[54]

- **NPM1 mutations**

✓ **Falini et al, 2005**

NPM1 mutations are seen in about 30% of adult AML. If there is no internal tandem duplication in FLT3, it is associated with a good prognosis .They are CD34 –ve and CD33+ve.[55]

- **AML with mutated CEBPA.**

✓ **Haferlach et al, 2009**

The most common aberrant antigen expression is CD7.[56]

CD7

In most of the studies, expression of CD was associated with poor outcome.

Chang et al, 2007

37% of AML were CD7 positive. CD7 expression was an independent risk factor. They were associated with shorter disease free survival .[57]

Tiftik et al, 2004; Suzuki et al, 2010

CD7 positive CD56 positive AML has a characteristic clinical feature. They present most often with lymphadenopathy with or without a mediastinal mass ,extramedullary involvement . They don't repond well to therapy.[58,59]

Veronica et al, 2008

Expression CD7 is more commonly seen in association with FLT3/ITD mutation.[60]

CD 56

Raspadori et al, 2001, 2002

CD56 is expressed by natural killer cells. Normal myeloid cells don't express CD56. It associated with bad prognosis like incomplete response to therapy and poor survival .[61]

Yang et al, 2007

Extramedullary infiltration is more common with CD56 expression . AML with t(8;21) usually has a favourable prognostics but if CD56 is expressed it is poor. [62]

Alegretti AP, Bittar CM, Bittencourt R, Piccoli AK, Schneider L, Silla LM, et al.,2011

Eight cases (16.7%) were CD56 positive without correlation to age or gender. The highest incidence of CD56 positivity was in FAB subtypes M4 and M5. The death rate during induction was not significantly different between patients with and without CD56 expression (62.5% vs. 27.5%; p-value = 0.097). However, patients that expressed CD56 had significantly lower overall survival than those who did not (mean 4.0 months vs. 14.5 months; p-value = 0.03).

Elyamany, G. et al (2013).

The prognostic significance of selected markers of cells is well known, CD7 and CD56 expression at diagnosis has been associated with low remission rates and biological aggressiveness in a significant proportion of acute leukemias .

Incidence of aberrant phenotypes

Zheng et al, 2008.

The leading lymphoid associated antigen was CD7(20.5%) followed by CD2, CD19 and CD22. Aberrant markers like CD8, CD5, CD10, CD20, CD3 and cCD79a were detected in lower than 5% of AML cases.[63]

Jahedi et al ,2014.

Aberrant phenotype was seen in 57.1% of AML cases. CD7 and CD2 were the most frequent lymphoid associated antigen found in the sample cases.CD7 was expressed in 72.7% of cases in M1 and 28.5% in M2 . CD7 was not expressed in any of the M3 and M4 cases. CD2 was most commonly seen in M1 followed by M2 and M3. CD2 was not seen in M4.[64]

Jha R et al, 2013.

Aberrant phenotype were seen in about 35% of cases. M7 did not show any aberrancy. All M0 cases had aberrant phenotype and about 51.9% in . Lymphoid associated antigen in descending order are CD 7(20%) ,CD 4(14%) ,CD 19(8%).[65]

Azza H. El-Sissy et al,2006.

CD9 (29.4%) was the leading expressed aberrant antigen followed by CD7,CD19, CD4 and CD22.[66] But according to **Shen et al., Bahia et al., John et al.**, CD7 was the most common aberrant antigens associated with AML.[67,68]

Nahla AB Abdulateef et al,2014.

Aberrant phenotype was detected in 67.5% of AML cases. There were no correlation between aberrant phenotype and prognosis but AML cases expressing two or more lymphoid associated antigens showed a lower median relapse free survival than those positive for a single aberrant antigen . CD7 was associated with an unfavorable cytogenetic pattern (p=0.046).[69]

Bahia et al,2001

A very high % of aberrant phenotypes , about 88.6% was found in the AML cases. Lymphoid associated antigen expression was about 34.3% . Asynchronous aberrant phenotype (82.4%) was the most common. CD7 was the most frequent lymphoid-associated antigen. The most frequent asynchronous aberrant phenotype was $CD117^{+}$ $CD34^{+/-}$ $CD11c^{+}$(67.6%) and $CD117^{+}CD34^{+/}$ $CD15^{+}$ (61.7%) of the cases, respectively. $CD117^{+}CD34^{+/}$ $CD15^{+}$ phenotype was associated with good phenotype a relevant association with clinical prognosis.[70]

Launder et al,1996.

Aberrant phenotype was seen in 22% AML cases. CD7(44%), CD2(38%), and CD5(25%) ,CD19(13%) and CD20(6%) were expressed. They suggested that expressed aberrant antigens CD7, CD5, and CD2 in otherwise straightforward AML should not be considered as lymphoid lineage commitment .[71]

Dexler HG et al,1993.

CD4 and CD7 antigens were expressed in 24% and 15% of AML cases respectively. CD7+AML appeared to be associated with poor prognosis.[72]

Arber et al ,1996.

CD79a is a B lymphocyte marker. CD79a was expressed by 5% of all non-APL AMLs and 90% of all APL studied. All APL had the characteristic t (15;1 7) (q24;q21). CD79a is commonly expressed in association with t (15;17) AML.[73]

Aberrancies and prognosis in AML

Immunophenotypic aberrancies have been explored to predict treatment outcome in AML. The results are not unequivocal.

- Some studies reported an adverse prognostic association with a multitude of markers (cytTdT, CD7, CD9, CD11b, CD13, CD14, CD33, CD34 and CD56), while others failed to show any association between immunophenotype and treatment outcome (Lee et al, 1992; Raspadori et al, 1997; Venditti et al,1998; Ogata et al, 2001; Be´ne´, 2005).
- Some antigens deserve further attention because their expression is related to survival.

INCIDENCE OF ABERRANT PHENOTYPES IN VARIOUS STUDIES

STUDY	ABERRANT PHENOTYPE %
Macedo A,1995	73%
Launder et al,1996.	22 %
Bahia.D.M et al,2001.	88 %
Nahla AB Abdulateef et al,2014.	67.5%
Azza H. El-Sissy et al,2006.	47%
Jha R et al, 2013.	35%
Naghmana Mazhar et al.2013	38%
Jahedi et al ,2014.	57.1%
Bhushan, Bharat, et al 2010	49%

MATERIAL AND METHODS

SELECTION OF CASES

The study conducted on freshly collected samples from 35 newly diagnosed acute myeloid leukemia patients . Acute myeloid leukemia was confirmed by bone marrow aspiration / biopsy and peripheral blood smear based on the morphology and immunophenotyping.

STUDY CENTRE

Institute of internal medicine and Department of hematology,
Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai

DURATION OF THE STUDY

6 months, from Feb 2014 to July 2014.

STUDY DESIGN

Cross sectional study

SAMPLE SIZE

35 patients

DATA COLLECTION AND METHODS

35 cases of newly diagnosed AML were selected according to the selection criteria. Immunophenotyping was done on fresh bone marrow aspirate or peripheral blood samples. We applied Acute Leukemia Panel with these following CD markers:
CD3, CD7, CD10, CD13, CD14, CD15, CD10, CD19, CD33, CD34,CD45,CD117.

CD marker was considered to be positive when more than 20% blast cells are positive.

INCLUSION CRITERIA

More than 20% myeloblast in bone marrow

EXCLUSION CRITERIA

1.Patient under treatment

2.Relapsed AML patients

INVESTIGATION DETAILS

Immunophenotyping :

Bone Marrow Samples

Bone marrow Samples were aspirated from the iliac crest. The specimens were collected and drawn into EDTA tube. Samples were processed within 1hour.

Peripheral blood samples

The patients WBC count was adjusted at less than 10x 10^3/ml. Each sample had more than mentioned count was diluted in order to reach the appropriate count. A specimen which contained any clout is excluded. A peripheral blood smear was prepared for microscopic assessment.

Bone marrow analysis

A bone marrow smear was prepared for microscopic examination. Bone marrow aspirate was checked for clot existence, if any was present, it was disintegrated with wooden sticks. In order to remove any fat or proteins, the bone marrow sample was washed before the procedure. Washing instruction steps are: adding about 4-5 ml of 2% PBS to 1.5 -2 ml of bone marrow aspirate in test tube, 10 minutes centrifuge at 2500 rpm , removing the supernatant and resuspending pellet with adequate volume of 2%PBS. These steps repeated two times

Flow Cytometric Analysis of Acute Leukemia Cases

Lysing and Staining:

The reagents and capped polystyrene test tubes were provided by Beckman coulter. Ten microliters of fluorescein isothiocyanate (FITC) conjugated monoclonal antibody, 10µl of Phycoerythrin (PE) conjugated monoclonal antibody, 5µl of Allophycocyanin (APC) conjugated monoclonal antibody, and 5µl of Peridinin-chlorophyll protein complex (perCP) conjugated monoclonal antibody was added to the tubes, afterward 100µL of whole blood / bone marrow was in added each tube. Monoclonal antibodies (Abs) used in this study included fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin chlorophyll-protein (Per-CP) ,Allophycocyanin (APC) labeled CD3, CD7, CD10, CD13, CD14, CD15,

CD10, CD19, CD33, CD34,CD45,CD117.The mixture was Vortexed tenderly and incubated about 45 minutes to 1 hour in the dark area at room temperature (20-25˚C). Two ml of 1X lysing buffer was added to incubated mixture. Then it was vortexed tenderly and incubated for 20 minutes in the dark area at room temperature again; after that Centrifuge at 500g for 5 minutes was done.

The supernatant was removed. Subsequently 2-3 ml of washing buffer was added and centrifuged at 500g for 5 minutes and the supernatant was removed. 1 ml of 1% cell fix (paraformaldehyde solution) was added and mixed completely, analysis can be done immediately or fixed cells can be stored at 2-8˚C until analysing them. Analysis was done by Beckman coulter brand flow cytometer. Samples vortexed thoroughly prior to acquisition. Abnormal populations were recognized by CD45/SSC gating, which was the base of calculating the positive rate of leukemia-related antigens expressed on the abnormal cells. Antigen expression was considered to be positive

when the percentage of positive blast cells was equal or greater than 20%. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed that particular phenotype.

STATISTICAL METHODS

Data were analyzed qualitatively and quantitatively by means of SPSS 14 (Statistical package for social sciences 14). Frequency and descriptive analysis

were used in all statistical process. The statistical significance value was chosen to be below 0.05.

SPONSORSHIP

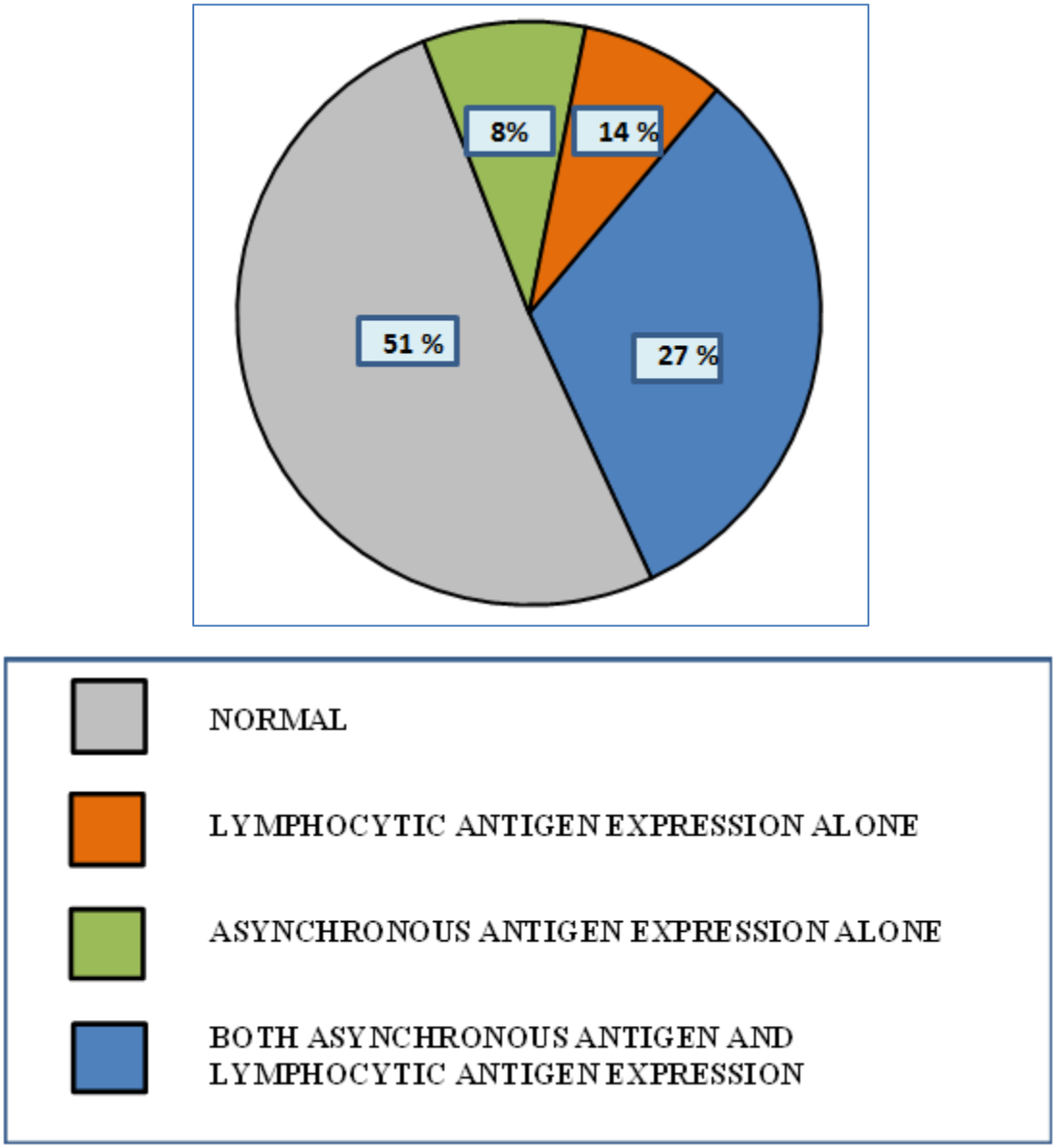
No

CONFLICT OF INTEREST

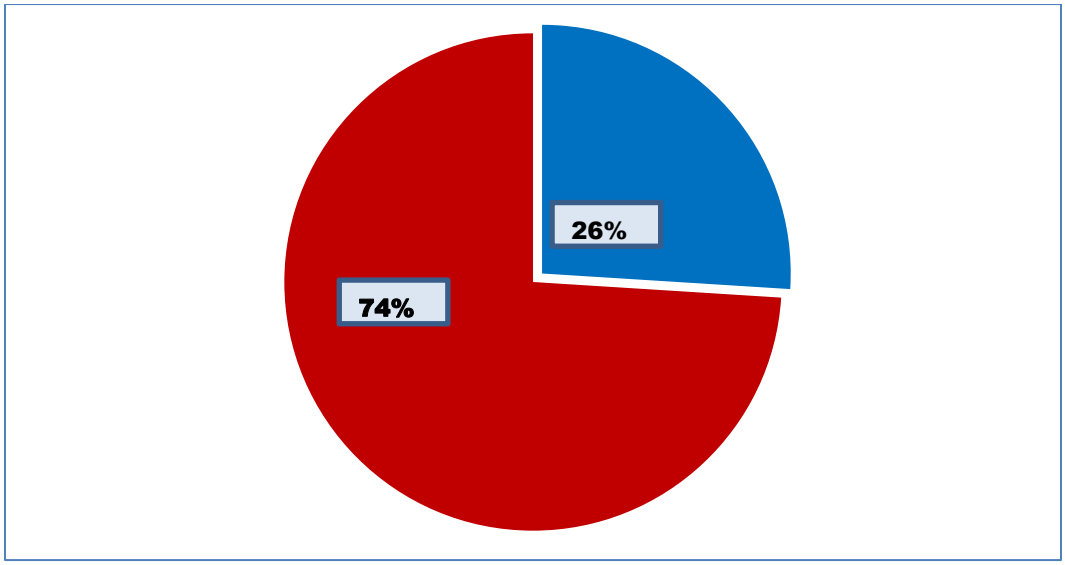
None

OBSERVATION AND RESULTS

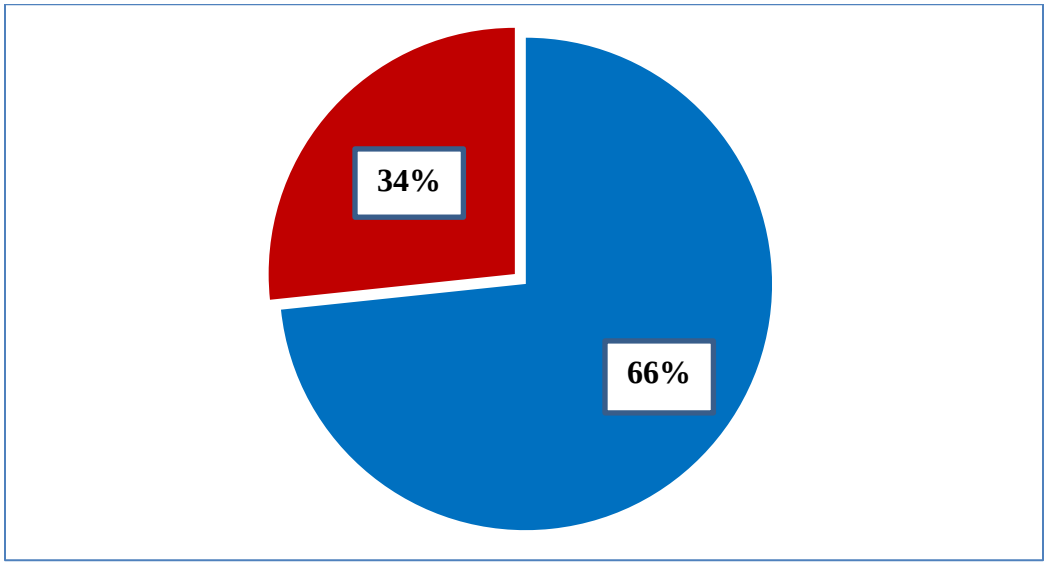
ABERRANT PHENOTYPE IN ACUTE MYELOID LEUKEMIA



Ly + /- AML IN MALES



Ly + /- AML IN FEMALES



POSTIVE RATE OF INDIVIDUAL ANTIGEN IN Ly+AML

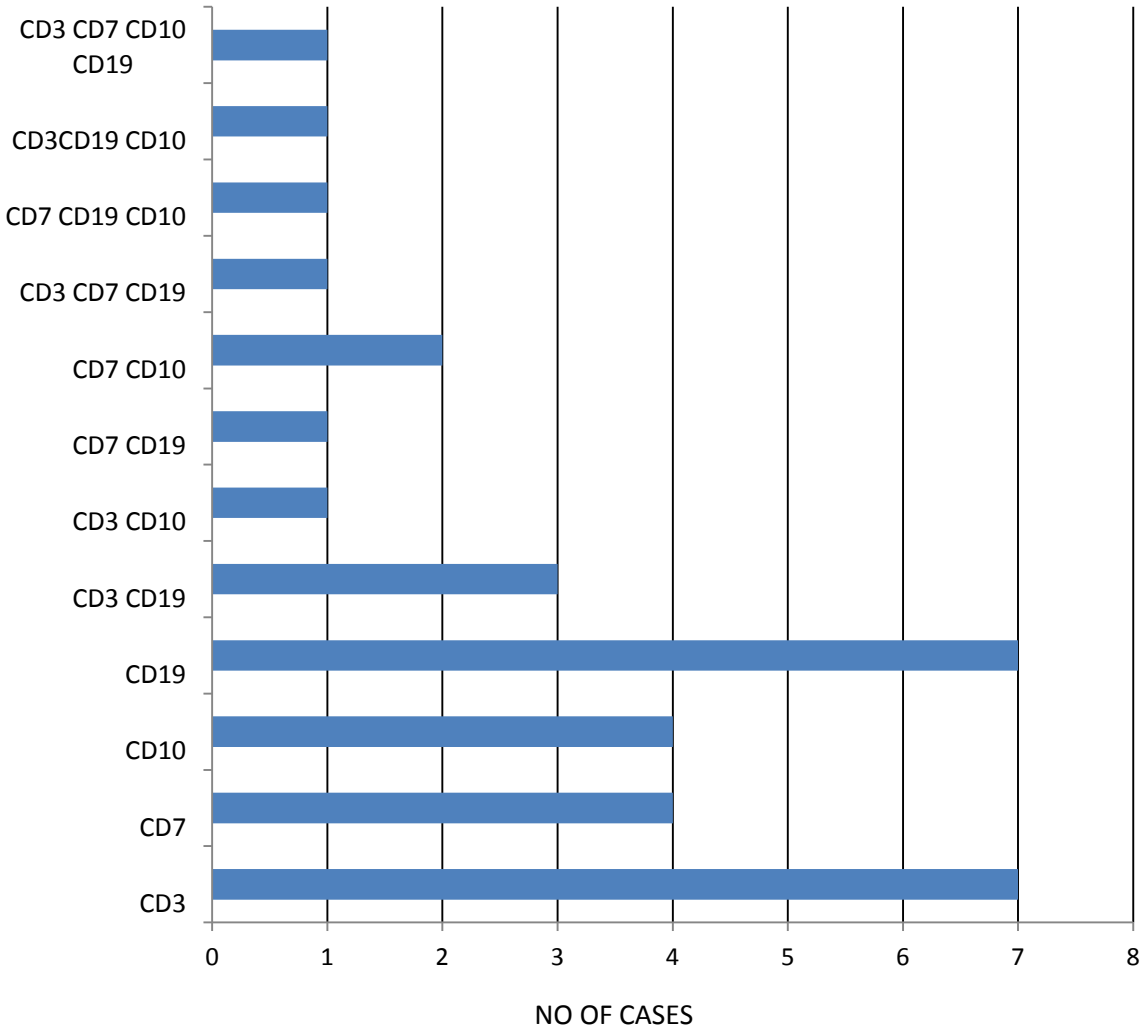
ANTIGEN	NO OF POSITIVE / NEGATIVE CASES	PERCENTAGE
CD3	7/35	20%
CD7	4/35	11.4%
CD10	4/35	11.4%
CD13	23/35	65.7%
CD14	6/35	17.1%
CD15	7/35	20%
CD19	7/35	20%
CD33	24/35	68.6%
CD 34	24/35	68.6%
CD117	16/35	20%

POSTIVE RATE OF INDIVIDUAL ANTIGEN IN Ly+AML

ANTIGEN	***n***	**%**
CD3	7/14	50%
CD7	4/14	28.6%
CD10	4/14	28.6%
CD13	9/14	64.3%
CD14	4/14	28.6%
CD15	4/14	28.6%
CD19	7/14	50%
CD33	10/14	71.4%
CD34	10/14	71.4%
CD117	10/14	71.4%

n = no of positive cases/no of negative cases
% = positive percentage

LYMPHOCYTIC ANTIGEN EXPRESSION



CD3 was the most common T cell antigen and CD19 was the most common B cell antigen expressed.

Distribution of aberrant T cell and B cell markers in AML

T & B Ly+AML	***n***	**Positive rate**
CD3	7/14	50%
CD7	4/14	28.6%
B Ly+AML		
CD19	7/14	50%
CD10	4/14	28.6%
Both T & B Ly+AML		
CD3,CD19	3/14	21.4%
CD3,CD10	1/14	7.1%
CD7,CD19	1/14	7.1%
CD7,CD10	2/14	14.3%
CD3, CD7,CD19	1/14	7.1%
CD7,CD19,CD10	1/14	7.1%
CD3,CD19,CD10	1/14	7.1%
CD3,CD7,CD10,CD19	1/14	7.1%

n = no of positive cases/no of negative cases

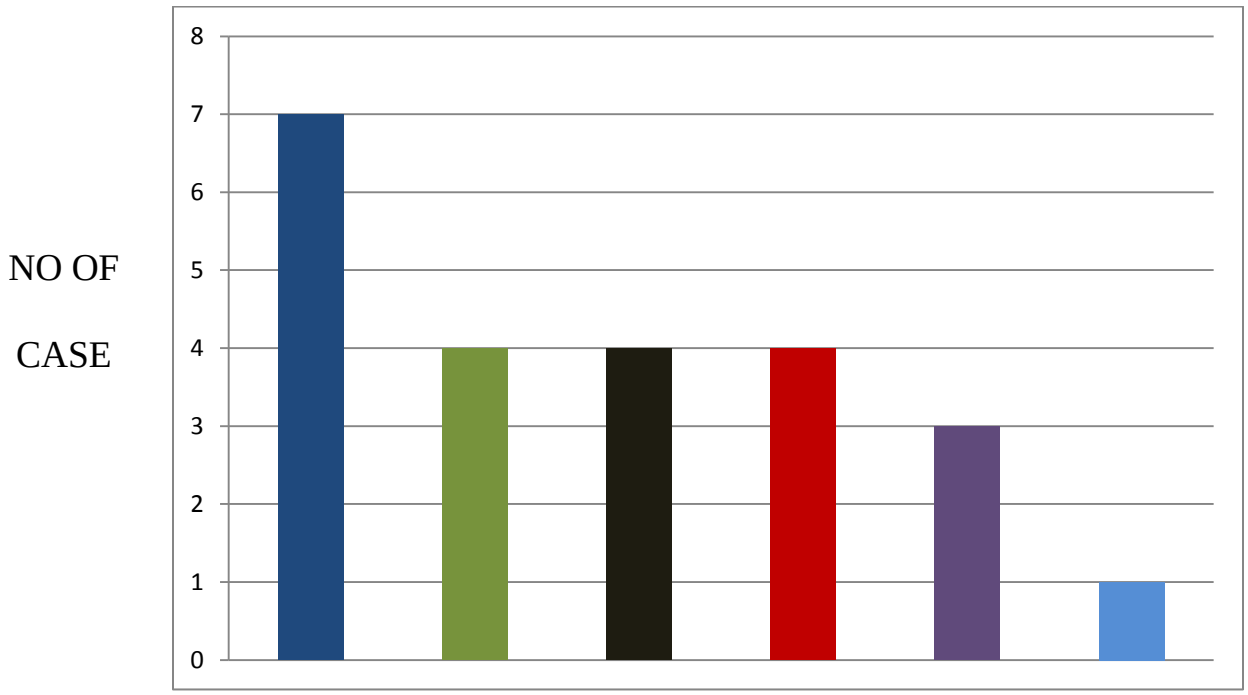
ASYNCHRONOUS ANTIGEN EXPRESSION IN AML

ASYNCHRONOUS ANTIGEN EXPRESSION	*n*	POSITIVE RATE
CD34+ CD15+	7/35	20%
CD34+ CD14+	4/35	11.4%
CD117+ CD34+ CD15+	4/35	11.4%
CD117+ CD34+ CD14+	4/35	11.4%
CD117+ CD34- CD15+	0/35	0%
CD117+CD34- CD14+	0/35	0%
CD117- CD34+ CD15+	3/35	8.6%
CD117- CD34+ CD14+	1/35	2.9%

n = no of positive cases/no of negative cases

Most common asynchronous antigen expression was CD34+CD15+.

Asynchronous antigen expression



Most common asynchronous antigen expression was CD34+CD15+.

CORRELATION OF IMMUNOPHENOTYPE AND POOR PROGNOSTIC FACTORS

ANTIGEN	AGE		MALE		WBC count >50000/mm^3		PLATELET COUNT<30000/mm^3		BLAST% >70%	
	n	%	*n*	%	*N*	%	*n*	%	*n*	%
CD3	0/7	0%	5/7	71.4%	2/7	28.6%	4/7	57%	4/7	57%
CD7	0/4	0%	1/4	25%	2/4	50%	2/4	50%	2/4	50%
CD10	1/4	25%	2/4	50%	¼	25%	2/4	50%	2/4	50%
CD13	2/23	8.7%	17/23	73.9%	17/23	73.9%	8/23	34.7%	13/23	56.5%
CD14	0/6	0%	3/6	50%	3/6	50%	3/6	50%	4/6	66.7%
CD15	0/7	0%	4/7	57.1%	2/7	28.6%	3/7	42.9%	5/7	71.4%
CD19	0/7	0%	2/7	28.6%	1/7	14.3%	5/7	71.4%	5/7	71.4%
CD33	2/24	8.3%	15/24	62.5%	6/24	25%	13/24	54.2%	10/24	41.7%
CD 34	1/24	4.2%	15/24	62.5%	6/24	25%	12/24	50%	12/24	50%
CD117	2/16	12.5%	10/16	62.5%	1/16	6.3%	5/16	31.3%	5/16	31.3%

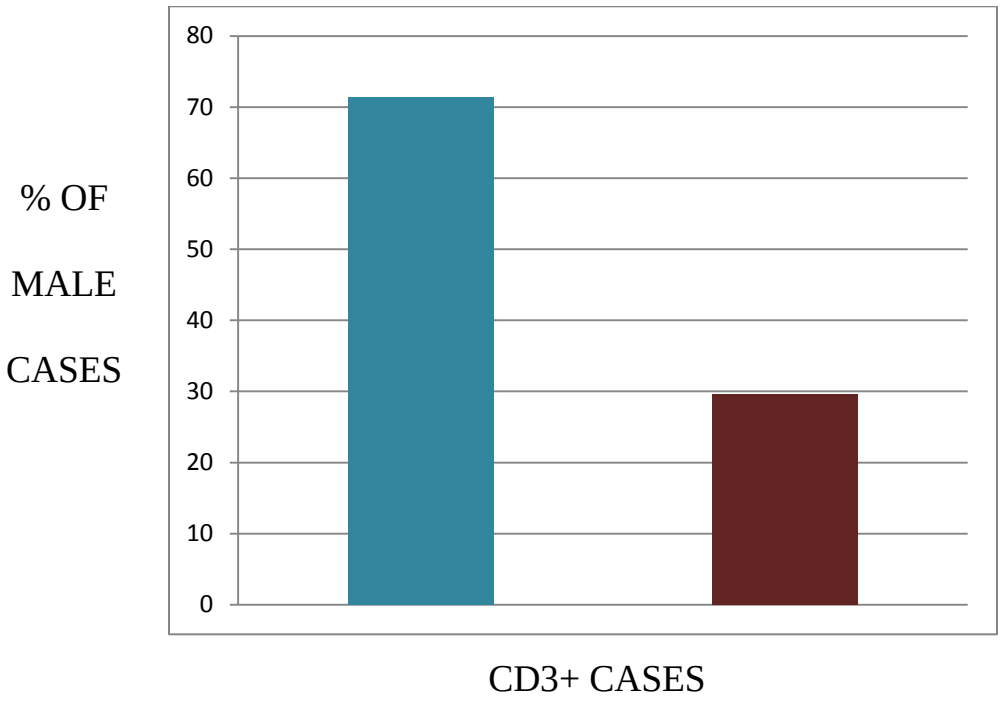
P VALUE =0.021

n = no of positive cases/no of negative cases

% = positive percentage

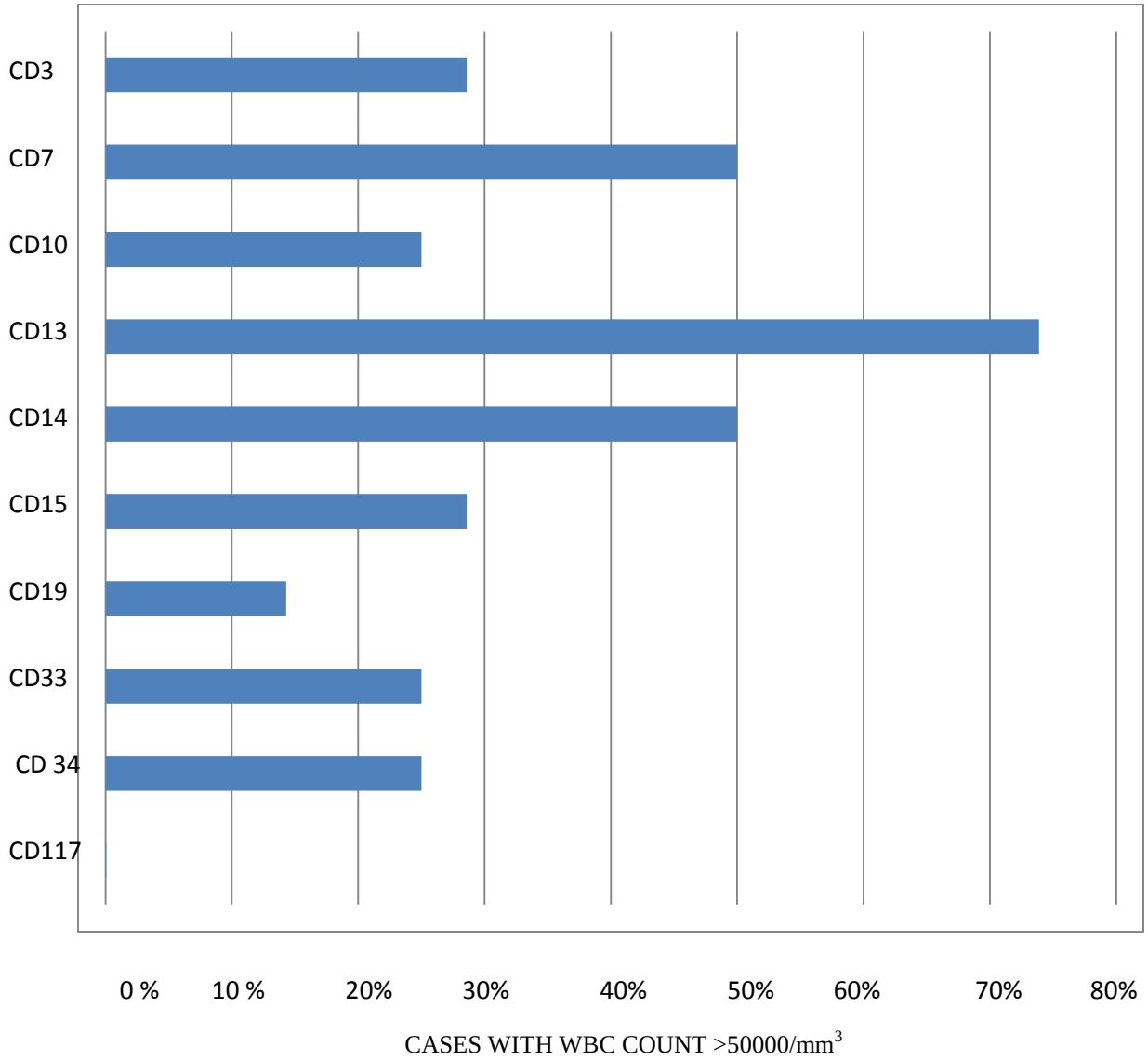
- CD3 was more commonly seen in male patients which was statistically significant (p=0.021)
- Statistically significant association could be made out between CD117 expression and low blast %

CD3 + CASES AND MALE GENDER



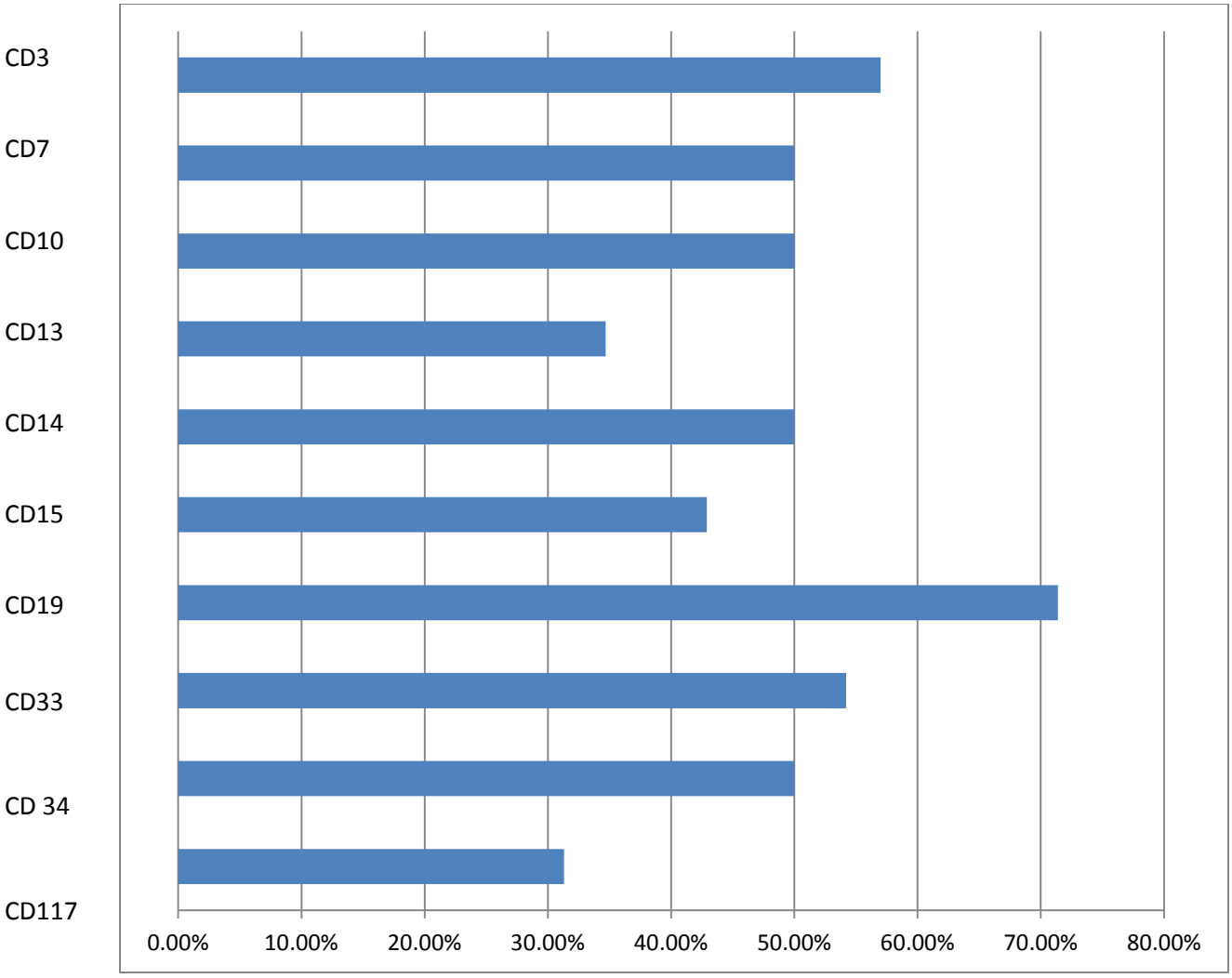
- CD3 was more commonly seen in male patients which was statistically significant (p=0.021)

CD MARKERS AND CASES WITH WBC COUNT >50000/mm^3



CD 7 had 50 % of the cases with WBC count more than 50000/mm^3 but it was not statistically significant.

CD MARKERS AND CASES WITH PLATELET COUNT >30000/mm^3



CD 3 and CD19 were the most common antigens expressed incases with platelet count less than 30000 but was not statistically significant.

Association of poor prognostic factors with aberrant phenotype in AML

Poor prognostic factors	Ly+AML		Ly-AML	
	n	**%**	***n***	**%**
Age >(60 yrs)	2/14	7.1%	1/21	9.5%
Gender (Male)	6/14	42.9%	17/21	81%
Wbc count > (50,000/mm3)	3/14	21.4%	6/21	28.6%
Platelet count < (30,000/mm3)	6/14	42.9%	9/21	42.9%
Blast % >(70%)	5/14	27.8%	13/21	72.2%

Ly + AML=lymphoid antigen positive AML,Ly-AML=Lymphoid antigen negative AML
n = no of positive cases/no of negative cases
% = positive percentage

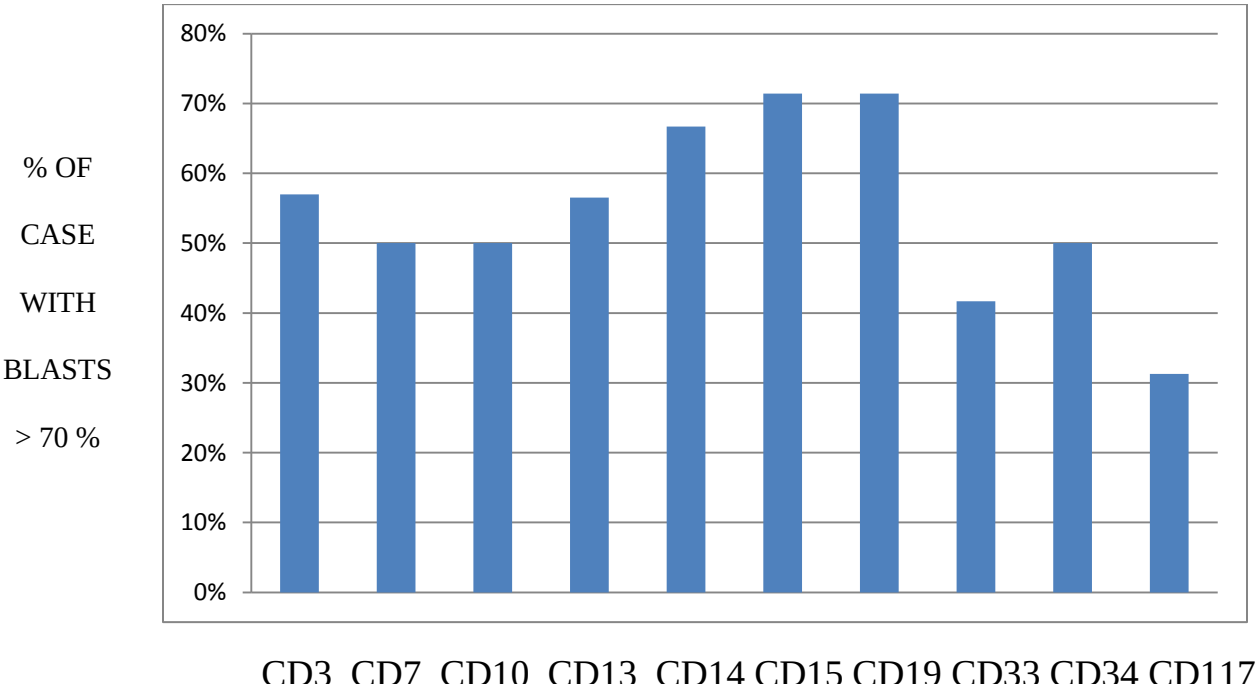
There were no statistically significant association between the Ly+ AML and poor prognostic factors.

PROGNOSTIC FACTORS (MEAN VALUE)

PROGNOSTIC FACTORS	**MEAN VALUE OF ALL CASES**
AGE(yrs)	40.65
WBC COUNT(/mm^3)	53979
PLATELET COUNT(/mm^3)	80114
BLAST %	62.65

PROGNOSTIC FACTORS	MEAN VALUE			
	CD3	CD7	CD10	CD19
AGE (yrs)	35.8	39	43	35.5
WBC COUNT(mm^3)	72328	54960	56975	45857
PLATELET COUNT(mm^3)	111142	61800	59250	69428
BLAST %	62.5	59.6	69.5	60.4

CD MARKERS AND BLAST % MORE THAN 70%



CD19 was the most common aberrant antigen having the blast% more than 70%.

POOR PROGNOSTIC FACTORS AND ASYNCHRONOUS ANTIGEN EXPRESSION

Poor prognostic factors	CD34+ CD15+		CD34+ CD14+	
	n	%	*N*	%
Age >(60 yrs)	0/7	0%	0/4	0%
Gender (Male)	4/7	5%	¼	25%
WBC count > (50,000/mm3)	3/7	42%	¼	25%
Platelet count < (30,000/mm3)	4/7	57%	2/4	50%
Blast % >(70%)	5/7	71%	¼	25%

n = no of positive cases/no of negative cases
% = positive percentage

There were no statistically significant association between asynchronous antigen expression and aberrant phenotypes.

DISCUSSION

INCIDENCE OF ABERRANT PHENOTYPE

- Of the 35 samples of newly diagnosed acute myeloid leukemia studied , 17 cases were of aberrant phenotype in our study. About 49 % of them had aberrant phenotype.
- The incidence of aberrant phenotype varied between different studies. Incidence rate ranging from 20 % to as high as 88 % have been reported in various studies.
- In a recent Study in Saudi Arabia in 40 AML patients aberrant antigens were present in 67.5%.[66] As high as 88% was reported by Bahia et al,2001.[70] Most of the recent studies have reported aberrant phenotype between 50% - 60% of cases , like Nahla AB Abdulateef et al,2014., Jahedi et al ,2014.[64,69]
- Low incidence around 20 % were reported only in few studies. Launder et al,1996. reported 22% of aberrant phenotypes. Jha R et al, 2013. Have reported 35% in their study.[65]

TYPE OF ABERRANT PHENOTYPE

- 5 cases had lymphoid associated antigen expression alone which is 14 % of cases. 3 cases had asynchronous antigen expression alone which is 8% of cases. 9 cases had both asynchronous antigen expression and lymphoid associated antigen expression which is 27 % of cases .

- In total lymphoid associated antigen expression is seen in 41 % of cases and asynchronous antigen expression in 35 % of cases.
- In our study lymphoid associated antigen expression was more slightly more common than asynchronous antigen expression. But in majority of the studies asynchronous antigen expression was the most common like 82 % in a study done by Bahia et al,2001.[70]

POSTIVE RATE OF INDIVIDUAL ANTIGEN IN ABERRANT PHENOTYPIC AML

- CD3 , CD19(lymphoid associated antigen) CD34+ CD15+ (asynchronous aberrant phenotype) were the most common equally expressed aberrant phenotypes, each 7 cases.
- CD 3 and CD 19 are the most common lymphoid associated antigen expressed. Each of them were expressed in 20 % of AML cases. About 7 cases out of 14 cases of Ly + AML expressed CD3 which is 50 %.Similarly CD19 was also expressed in 50% of the cases Ly + AML .
- CD 7 was seen in 4 cases , 28.6% of Ly + AML aberrant phenotypic cases. In most studies CD7 was the most common lymphoid associated antigen expressed , like Bahia et al, Jha R et al, 2013.,Zheng et al, 2008., Chang et al, 2007.[57,63,70,65]

- Like our study few studies have shown CD19 as the most common lymphoid associated antigen expressed. In a study done by Bhushan, Bharat et al, 2010, the revealed 32 % CD19 expression which the most common aberrant antigen and it was expressed more commonly that CD7 (15%).[74] In a study done by Jha R et al, 2013 CD19 expression was 8% lower compared to 20% in CD7 .
- CD34+ CD15+ was seen in 20 % cases AML cases. Incidence of CD34+ CD15+ was low when compared to the previous studies which have reported 61.5% of CD34+ CD15+ AML cases (Bahia et al , 2001).
- In majority of studies expression of early stem cell antigens, CD34 and CD117 with mature myeloid antigens was the most common aberrant change , like in study by haase et al, macedo et al,wells et al.[75-77]
- CD117+ CD34+ CD14+ was in 4 cases about 11.4% .

Aberrant phenotypes and its association with known adverse prognostic factors.

- Association of adverse prognostic factors and asynchronous antigen expression was studied.
- Adverse prognostic factors which were compared between Ly+ AML and Ly- AML groups.

- The adverse prognostic factors studied were Age (>60 yrs),Gender(male), WBC count (>50,000/mm3), Platelet count(<30,000/mm3), peripheral blast % (>70)
- In the total study population average age was 40.65 years, average WBC count was about 53979/mm^3, average platelet count was about 80114/mm^3 and average blast % was 62.65%.

Ly + AML and its association with known adverse prognostic factors.

CD3 + Ly+ AML

- 20 % of the AML cases were CD3 +.
- In CD3 + cases average age was 35.8 years , average WBC count about 72328 / mm^3, average platelet count was about 111142 /mm^3 and average blast % was 62.5%. There was no significant difference between the CD3+Ly+ AML and Ly – AML in average WBC count platelet count and blast %.
- In CD3 + Ly+ AML males were (5/7)71.4% , WBC count (>50,000/mm3) were (2/7)28.6%, Platelet count(<30,000/mm3) were (4/7)57%, peripheral blast % (>70) were (4/7)57%. CD3 was more commonly seen in male patients which was statistically significant (p=0.021)

CD7 + Ly+ AML

- In CD7 + cases average age was 39 years , average WBC count about 54960/mm^3, average platelet count was about 61800 /mm^3 and average blast % was59.6% .There were no significant between between CD7+Ly+AML and Ly- AML in terms of average age , WBC count , platelet count and blast %.
- In CD7+Ly+AML males were (1/4)25%, WBC count (>50,000/mm3) were (2/4)50%, Platelet count (<30,000/mm3) were (2/4)50%, peripheral blast % (>70) were (2/4)50%. There were no significant association between CD7+Ly+AML and Ly- AML.
- In few studies CD7+ AML was assoiciated with poor prognosis like lower response rate than CD7- AML patients(Hurwitz et al)[78]

CD10 + Ly+ AML

- In CD10+ cases average age was 43 years , average WBC count about 56975/mm^3, average platelet count was about 59250 /mm^3 and average blast % was 69.5% . There were no significant between between CD10+Ly+AML and Ly- AML in terms of average age , WBC count , platelet count and blast %.

- In CD10 + Ly+ AML males were (2/4)50% , WBC count (>50,000/mm3) were (1/4)25%, Platelet count (<30,000/mm3) were (2/4)50%, peripheral blast % (>70) were (2/4)50%. There were no significant association between CD10+Ly+AML and Ly- AML.

CD19 + Ly+ AML

- In CD19 + cases average age was 35.5 years , average WBC count about 45857/mm^3, average platelet count was about 69428 /mm^3 and average blast % was 60.4% .
- In CD19 + Ly+ AML males were (2/7)28.6% , WBC count (>50,000/mm3) were (1/7)14.3% , Platelet count (<30,000/mm3) were (5/7)71.4%, peripheral blast % (>70) were (5/7)71.4% . Even though there is no statistically significant association with adverse prognostic factors , the percentage of cases with blast % more than 70% was about 71.4 % which is high.(p=0.176). The chi-square statistic for platelet count < 30000/mm^3 was 2.917. The P-Value is 0.08.

BOTH B AND T CELL Ly + AML

- CD3+,CD19+ was seen in 3/14 cases 21.4%.CD7+,CD19+ was seen in 2/14 cases 14.3%. Both T cell and B cell Ly + AML was less compared with T cell marker alone or B cell marker alone expression.

ASYNCHRONOUS ANTIGEN EXPRESSION AND ITS ASSOCIATION WITH POOR PROGNOSTIC FACTORS

- In CD34+CD15+ patients 57% were males, 42 % had WBC count > 50000/mm^3,57% had platelet count less than 30000/$mm^3$and 71 % of them had blast % more than 70%.
- CD34+CD1+ patients 25% were males, 25 % had WBC count > 50000/mm^3,50% had platelet count less than 30000/$mm^3$and 25 % of them had blast % more than 70%.

LIMITATIONS OF STUDY

- One limitation of this study is low number of patients less than 60 yrs of age
- The results should be confirmed in large group of patients.

CONCLUSION

There is a frequent occurrence of aberrant phenotype in acute myeloid leukemia in India like in other majority of studies . CD19 and CD3 were the most commonly expressed lymphoid associated antigen. Lymphoid associated expression were slightly more common than asynchronous antigen expression. Most common asynchronous aberrant phenotype was CD34+CD15+. Aberrant phenotypic expression were not associated with poor risk factors in acute myeloid leukemia except for common expression of CD3 in males.

BIBLIOGRAPHY

1.Preston DL, Kusumi S, Tomonaga M, et al: Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950–1987. Radiat Res 137(2 Suppl):S68, 1994.

2. Lichtman MA: Is there an entity of chemically induced BCR-ABL-positive chronic myelogenous leukemia? Oncologist 13:645, 2008. [PMID: 18586919]

3.Brownson RC, Novotny TE, Perry MC: Cigarette smoking and adult leukemia: A meta-analysis.Arch Intern Med 153:469, 1993. [PMID: 8435026]

4.van Rhenen A, van Dongen GA, et al: The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood 110:2659, 2007.

5.Look AT: Oncogene transcription factors in human acute leukemias. Science 278:1059, 1997. [PMID: 9353180]

6.Grimwade D, Enver T: Acute promyelocytic leukemia: Where does it stem from? *Leukemia* 18:375, 2004. [PMID: 14737069]

7.Zeisig BB, Kwok C, Zelent A, et al: Recruitment of RXR by homotetrameric RARalpha fusion proteins is essential for Transformation. *Cancer Cell* 12:36, 2007. [PMID: 17613435]

8.Sipp N, Radaszkiemicz T, Meijer CJLM, et al: Specific skin manifestations in acute leukemia with monocytic differentiation.

9.Cancer 71:124, 1993.Kincaid MC, Green WR: Ocular and orbital involvement in leukemia. Surv Ophthalmol 27:211, 1983. [PMID: 6342189]

10. Lisker SA, Finkelstein D, Brody JI, Beizer LH: Myocardial infarction in acute leukemia. Arch Intern Med 119:332, 1967.

11.Pavlovsky S, Eppinger-Helft M, Murill FS: Factors that influence the appearance of central nervous system leukemia. Blood 42:935, 1973. [PMID: 4519105]

12.Wiernik PH: Acute leukemias, in DeVita VTJr, Hellman S, Rosenberg SA ledsl: Cancer: Principles and Practice of Oncology, ed 3. Philadelphia, JB Lippincott Co, 1989, pp 1809-1835.

13. Grimwade, D. et al. (2001) The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood, 98, 1312–1320 2 Smith, M.A. et al. (1996) The secondary leukemias: challenges and research directions. J Natl Cancer Inst, 88, 407–418.

14. Paschka P, Du J, Schlenk RF, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML study group (AMLSG). Blood 2013;121:170-177.

15. Kainz B, Heintel D, Marculescu R, et al. Variable prognostic value of FLT3 internal tandem duplications in patients with de novo AML and a normal

karyotype, t(15;17), t(8;21) or inv(16). Hematol J 2002;3:283-289. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12522450.

16. Whitman SP, Maharry K, Radmacher MD, et al. FLT3 internal tandem duplication associates with adverse outcome and gene- and microRNA-expression signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood 2010;116:3622-3626. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20656931.

17. Barjesteh van Waalwijk van Doorn-Khosrovani S, Erpelinck C, Meijer J, et al. Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. Hematol J 2003;4:31-40. Available at:http://www.ncbi.nlm.nih.gov/pubmed/12692518.

18. Green CL, Koo KK, Hills RK, et al. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations. J Clin Oncol 2010;28:2739-2747. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20439648

19.Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood 2002;100:2717-2723. Available at:http://www.ncbi.nlm.nih.gov/pubmed/12351377.

20.Byrd et al, 2002, “Pretreatment cytogenetic abnormalities are predictive of

induction success, cumulative incidence of relapse, and overall survival in adult patients with denovo AML: results from Cancer and Leukemia Group B," Blood, 100, 4325-4336.

21. Tejinder Singh, "Acute leukemias", Chapter 6 in Atlas and text book of Hematology, 1st edition, Avichal Publishing Company, 2010, 143.

22. Jine Zheng et al, 2008, "A correlation study of immunophenotypic, cytogenetic and clinical features of 180 AML patients in China." Cytometry Part B, 74B, 25-29.

23. Grimwade.D et al, 1998, "The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial." Blood, 92, 2322-2333.

24. Bennett JM, Catovsky D, Daniel MT, et al: Proposed revised criteria for the classification of acute myeloid leukemia: A report of the French-American-British Group. Ann Intem Med 1985; 103:460-462.

25.Arber.D.A et al, 2003, "Prognostic impact of AML classification. Importance of detection of recurring cytogenetic abnormalities and multilineage dysplasia on survival." American Journal of Clinical Pathology, 119, 672-680.

26.Garand.R et al, 1995, "Minimally differentiated erythroleukemia (AML M6'variant'): a rare subset of AML distinct from AML M6. Groupe Francais d Hematologie Cellulaire. *British Journal of Haematology*, 90, 868-875.

26.O K Weinberg and D A ArberMixed-phenotype acute leukemia:

historical overview and a new definition leukemiaISSN: 0887-6924 EISSN: 1476-5551

27. Golomb HM, Rowley JD, Vardiman J, et al: "Microgranular" acute promyelocytic leukemia: A distinct clinical, ultrastructural, and cyto-genetic entity. *Blood* 55:253, 1980. [PMID: 6928105]

28. McKenna RW, Parkin J, Bloomfield C, et al: Acute promyelocytic leukaemia: A study of 39 cases with identification of a hyperbasophilic microgranular variant. *Br J Haematol* 50:201, 1982. [PMID: 6949609]

29. Rovelli A, Biondi A, Rajnoldi AC, et al: Microgranular variant of acute promyelocytic leukemia in children. *J Clin Oncol* 10:1413, 1992. [PMID: 1517784]

30. Menell JS, Cesarman GM, Jacovina AT, et al: Annexin II and bleeding in acute promyelocytic leukemia. *N Engl J Med* 340:994, 1999. [PMID: 10099141]

31.Huhn D, Twardzik L: Acute myelomonocytic leukemia and the French-American-British classification. *Acta Haematol* 69:36, 1983. [PMID: 6401891]

32. Cuneo A, VanOrshoven A, Michaux JL, et al: Morphologic, immunologic and cytogenetic studies in erythroleukemia: Evidence for multilineage involvement and identification of two d.istinct cytogenetic clinicopathologic types. *Br J Haematol* 75:346, 1990. [PMID: 2386768]

33. Goldberg SL, Noel P, Klumpp TR, Dewald GW: The erythroid leukemias. *Am*

J Clin Oncol 21:42, 1998. [PMID: 9499256]

34.Manoharan A, Horsley R, Pitney WR: The reticulin content of bone marrow in acute leukemia in adults. Br J Haematol 43:185, 1979. [PMID: 508627]

35. Gabbas AG, Li CF: Acute non-lymphocytic leukemia with eosinophilic differentiation. *Am J Hematol* 21:29, 1986. [PMID: 3706287]

36. Brito-Babapulle F: Clonal eosinophilic disorders and the hypereosinophilic syndrome. *Blood Rev* 11:129, 1997. [PMID: 9370044]

37. Mezger J, Permanetter W, Gerhartz H, et al: Philadelphia chromosome-negative acute hematopoietic malignancy: Ultrastructural, cytochemical, and immunocytochemical evidence of mast cell and basophil differentiation. *Leuk Res* 14:169, 1990. [PMID: 2107368]

38. Lichtman MA, Segel GB: Uncommon phenotypes of acute myelogenous leukemia: Basophilic, mast cell, eosinophilic, and myeloid dendritic cell subtypes: A review. *Blood Cells Mol Dis* 35:370, 2005. [PMID: 16203163]

39.National Comprehensive Cancer Network. NCCN Practice Guidelines in Oncology: Acute Myeloid Leukemia. V.2.2014.

40.Lo-Coco F, Avvisati G, Vignetti M, et al.Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med. 2013 Jul 11;369(2):111-21.

41.Powell BL, Moser B, Stock W, et al. Arsenic trioxide improves event-free and overall survival for adults with acute promyelocytic leukemia: North American

Leukemia Intergroup Study C9710. Blood. Nov 11 2010;116(19):3751-7.

42. Fenaux P, Chastang C, Chevret S, et al. A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. The European APL Group. Blood. Aug 15 1999;94(4):1192-200.

43.Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia.J Clin Oncol 2003;21(24):4642-4649.

44.Cheson BD, Bennett JM, Kopecky KJ, et al. International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia: revised recommendations of the International Working Group for diagnosis, standardizations of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003;**21**(24):4642-4649.

45. Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982

46.Campana D, Coustan-Smith E. Detection of minimal residual disease in acute

leukemia by flow cytometry. Cytometry. 1999;38:139.

47.Campana D, Pui C-H. Detection of minimal residual disease in acute leukemia: methodological advances and clinical significance. Blood 1995;**85**(6):1416-1434.

48.Campana D. determination of minimal residual disease in leukemia patients. Br J Haematol 2003;**121**(6):823-838

49.Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. Lancet Oncol 2010;**11**(6):543-552.

50. Terstappen, L. W., et al. "Flow cytometric characterization of acute myeloid leukemia. Part 1. Significance of light scattering properties." Leukemia 5.4 (1991): 315-321.

51.Khoury, H., Dalal, B.I., Nevill, T.J., Horsman, D.E., Barnett, M.J., Shepherd, J.D., Toze, C.L., Conneally, E.A., Sutherland, H.J., Hogge, D.E. & Nantel, S.H. (2003) Acute myelogenous leukemia with t(8;21) – identification of a specific immunophenotype. Leukaemia & Lymphoma, 44, 1713–1718.

52.Chen SW, Li CF, Chuang SS, Tzeng CC, Hsieh YC, Lee PS, Chen CH, Huang WT, Hwang WS, Tsao CJ. Aberrant co-expression of CD19 and CD56 as surrogate markers of acute myeloid leukemias with t(8;21) in Taiwan. Int J Lab Hematol. 2008 Apr;30(2):133-8.

53. Dunphy, C.H. (1999) Comprehensive review of adult acute myelogenous leukemia: cytomorphological, enzyme cytochemical, flow cytometric

immunophenotypic, and cytogenetic findings. Journal of Clinical Laboratory Analysis, 13, 19–26.

54.Paietta, E., Goloubeva, O., Neuberg, D., Bennett, J.M., Gallagher, R., Racevskis, J., Dewald, G., Wiernik, P.H. & Tallman, M.S. (2004) Eastern Cooperative Oncology Group. A surrogate marker profile for PML/RAR alpha expressingacute promyelocytic leukemia and the association of immunophenotypic markers with morpho logic and molecular subtype. Cytometry. Part B, Clinical Cytometry, 59, 1–9.

55.Falini, B., Mecucci, C., Tiacci, E., Alcalay, M., Rosati, R., Pasqualucci, L., La Starza, R., Diverio, D., Colombo, E., Santucci, A., Bigerna, B., Pacini, R., Pucciarini, A., Liso, A., Vignetti, M., Fazi, P., Meani, N., Pettirossi, V., Saglio, G., Mandelli, F., Lo-Coco, F., Pelicci, P.G. & Martelli, M.F. (2005) Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. New England Journal of Medicine, 352, 254–256.

56.Haferlach, C., Mecucci, C., Schnittger, S., Kohlmann,A., Mancini, M., Cuneo, A., Testoni, N.,Rege-Cambrin, G., Santucci, A., Vignetti, M., Fazi, P., Martelli, M.P., Haferlach, T. & Falini, B.(2009) AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. Blood, 114, 3024–3032.

57. Chang, H., Yeung, J., Brandwein, J. & Yi, Q. (2007) CD7 expression predicts poor disease free survival and post-remission survival in patients

with acute myeloid leukemia and normal karyotype. Leukemia Research, 31, 157–162.

58. Tiftik, N.Z., Bolaman, S., Batun, O., Ayyildiz, A., Isikdogan, A., Kadikoylu, G. & Muftuoglu, E. (2004) The importance of CD7 and CD56 antigens in acute leukaemias. International Journal of Clinical Practice, 58, 149–152

59. Suzuki, R., Ohtake, S., Takeuchi, J., Nagai, M.,Kodera, Y., Hamaguchi, M., Miyawaki, S., Karasuno, T., Shimodaira, S., Ohno, R., Nakamura, S. & Naoe, T. (2010) The clinical characteristics of CD7+ CD56+ acute myeloid leukemias other than M0. International Journal of Hematology, 91, 303–309

60.Veronica Rausei-Mills, MD, Karen L. Chang, MD, Karl K. Gaal, MD, Lawrence M. Weiss, MD, and Qin Huang, MD, PhD. Aberrant Expression of CD7 in Myeloblasts Is Highly Associated With De Novo Acute Myeloid Leukemias With FLT3/ITD Mutation ,Hematopathology.

61. Raspadori, D., Damiani, D., Michieli, M., Stocchi,R., Gentili, S., Gozzetti, A., Masolini, P.,Michelutti, A., Geromin, A., Fanin, R. & Lauria,F. (2002) PGP expression in acute myeloid leukemia: impact on clinical outcome. Haematologica, 87, 1135–1140.

62.Yang, D.H., Lee, J.J., Mun, Y.C., Shin, H.J., Kim, Y.K., Cho, S.H., Chung, I.J., Seong, C.M. & Kim, H.J. (2007) Predictable prognostic factor of CD56 expression in patients with acute myeloid leukemia with t(8:21) after high dose cytarabine or allogeneic hematopoietic stem cell transplantation. American Journal of Hematology, 82, 1–5.

63.Jine Zheng,Xingbing Wang,Yu Hu,Jing Yang,Jun Liu,Yanli He,Qing Gong, Junxia Yao,Xiaoqing Li,Wen Du,and Shiang HuangA Correlation Study of Immunophenotypic,Cytogenetic, and Clinical Features of 180 AML Patients in China. Cytometry Part B (Clinical Cytometry) 74B:25–29 (2008) q 2007

64.Mehdi Jahedi, Karim Shamsasenjan, Zohreh Sanaat, MohamadReza Aliparasti, Shohreh Almasi, Mozhdeh Mohamadian1, Babak Nejati, Amir Kamalifar, Ali Akbar Movassaghpour .Aberrant Phenotype in Iranian Patients with Acute Myeloid Leukemia Advanced Pharmaceutical Bulletin, 2014, 4(1), 43-47

65.Jha R, Grover G, Bose P.Lymphoid associated antigen expression in new cases of Acute Myeloid Leukemia .Journal of Pathology of Nepal (2013) Vol. **3**, 487-490

66.AZZA H. EL-SISSY, M.D.; MAY A. EL-MASHARI, M.D.; WAFAA Y. BASSUNI, M.D. and AZIZA F. EL-SWAAYED, M.D. Aberrant Lymphoid Antigen Expression in Acute Myeloid Leukemia in Saudi Arabia .Journal of the Egyptian Nat. Cancer Inst., Vol. 18, No. 3, September: 244-249, 200616.

67.Shen HQ, Tang YM, Yang SL, Qian BQ, Song H, Shi SW, et al. Immunophenotyping of 222 children with acute leukemia by multi-color flow cytometry. Zhonghua Er Ke Za Zhi. 2003, 41 (5): 334-7.

68.John L Frater, Nabeel R Yaseen, LoAnn C, et al. Biphenotypic acute leukemia with coexpression of CD79a and markers of myeloid lineage. Archi PatholLab Med. 127 (3): 356-9.

69. Nahla Ahmad Bahgat Abdulateef, Manar Mohammad Ismail, Hanadi

Aljedani.Clinical Significance of Co-expression of Aberrant Antigens in Acute Leukemia: A Retrospective Cohort Study in Makah Al Mukaramah, Saudi Arabia. Asian Pac J Cancer Prev, **15 (1)**, 221-227

70.DM, Yamanmoto M, Chauffaille ML, Kimura EY, Bordin JO, Fliqueiras A, et al. Aberrant phenotypes in acute myeloid leukemia: Ahigh frequency and its clinical significance. Hematologica. 2001, 86: 801-6.

71.Launder TM, Bray RA, Stempora L, Chenggis ML, Farhi DC. Lymphoid-associated antigen expression by acute myeloid leukemia.Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia 30322, USA. Am J Clin Pathol. 1996 Aug;106(2):185-91.

72.Drexler HG, Thiel E, Ludwig WD. Acute myeloid leukemias expressing lymphoid-associated antigens:diagnostic incidence and prognostic significance. Leukemia. 1993 Apr;7(4):489-98.

73.Daniel A. Arber, Kay A. Jenkins, and Marilyn L. Slovak .CD79a Expression in Acute Myeloid Leukemia High Frequency of Expression in Acute Promyelocytic Leukemia.AmiericanJolornal of Patholoqy, Vol. 149, A'o. 4, October 1996 Short Communication.

74.Bharat Bhushan ,Pradeep Singh Chauhan , Sumita Saluja ,Saurabh Verma, Ashwani Kumar Mishra , Saeed Siddiqui , Sujala Kapur .Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome. Clin Exp Med (2010) 10:33–40

75.Wells SJ, Bray RA, Stempora LL, Farhi DC. CD117/CD34 expression in

leukemic blasts. Am J Clin Pathol
1996;106:192-5.
76. Macedo A, Orfao A, Vidriales MB, Lopex-Berges MC, et al. Characterization of aberrant phenotypes in acutemyeloblastic leukemia. Ann Hematol 1995;70:189-94.
77. Haase D, Feuring-Buske M, Schefer C, Schoch C, et al. Cytogenetic analysis of CD34+ subpopulations in AML andMDS characterized by the expression of CD38 and CD117. Leukemia 1997;11674-679.
78. Hurwitz CA, Raimondi SC, Head D, Krance R, et al. Distinctive immunophenotypic features of t(8;21)(q22;q22) acute myeloblastic leukemia in children. Blood 1992;80(12):3182-8.

PROFORMA

NAME OF THE PATIENT :

AGE / SEX :

IP/OP NUMBER :

OCCUPATION :

ADDRESS :

CONTACT NUMBER :

COMPLAINTS :

PAST HISTORY :

TREATMENT HISTORY :

DRUG ALLERGY :

GENERAL EXAMINATION :

VITALS :

SYSTEMIC EXAMINATION :

CARDIOVASCULAR SYSTEM :

RESPIRATORY SYSTEM :

ABDOMEN :

CENTRAL NERVOUS SYSTEM :

COMPLETE BLOOD COUNT:

PERIPHERAL SMEAR:

BONE MARROW ASPIRATION:

PROGNOSTIC FACTORS	
AGE(yrs)	
WBC COUNT(/mm^3)	
PLATELET COUNT(/mm^3)	
BLAST %	

FLOWCYTOMETRIC IMMUNOPHENOTYPING

ANTIGEN	POSITIVE / NEGATIVE	PERCENTAGE
CD3		
CD7		
CD10		
CD13		
CD14		
CD15		
CD19		
CD33		
CD 34		
CD117		

INFORMATION SHEET

Study Title	:	Aberrant phenotypes in acute myeloid leukemia in India.
Study Centre	:	Rajiv Gandhi Government General Hospital, Chennai.
Patient's Name/Age	:	
Investigators Name	:	
Identification Number	:	

We are conducting a study on "**Aberrant phenotypes in acute myeloid leukemia in India**" among patients attending Rajiv Gandhi Government General Hospital, Chennai . You are being asked to participate in this study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns. Acute myeloid leukemia is a type of blood cancer The purpose of this study is to study about aberrant phenotypes in acute myeloid leukemia using flowcytometricimmunophenotyping and cytogenetics.Bone marrow

aspiration about 3 ml and peripheral blood about 3 ml will be collected from the patients for the study. There are no risks to the participating patients in the study .The result of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefits to future patients. The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared. Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time .Your decision will not result in any loss of benefits to which you are otherwise entitled.The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator

Signature of Participant

Date

PATIENT CONSENT FORM

Study Detail	:	Aberrant phenotypes in acute myeloid leukemia in India
Study Centre	:	Institute of Internal Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai.
Patient's Name	:	
Patient's Age	:	
Identification Number	:	

Patient may check (☑) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I

am free to withdraw at any time without giving reason, without my legal rights being affected.	☐
I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.	☐
I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.	☐
I hereby consent to participate in this study.	☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including haematological, biochemical, radiological tests. ☐

Signature/thumb impression

Patient's Name and Address:

Signature of Investigator

Study Investigator's Name:

Dr. A.THELENGANA

MASTER CHART

NO	NAME	IP NO	AGE	SEX	TOTAL WBC COUNT	PLATELET COUNT	BLAST %	CD3	CD7	CD10	CD13	CD14	CD15	CD19	CD33	CD34	CD 117	Ly
1	SELVARAJ.K	792	65	M	3400	16000	20	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	Ly-
2	MADHESH.G	12205	17	M	5000	43000	20	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	Ly-
3	DEVAPRIYA	18053	20	F	148300	30000	88	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	Ly+
4	LATHA	16770	22	F	22100	18000	60	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Ly+
5	SELVARAJ	124617	50	M	30300	53000	60	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Ly+
6	SHANKAR	50904	35	M	11000	88000	40	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	Ly-
7	BAKYARAJ	5192	33	M	205900	53000	90	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Ly-
8	JEVANANTHAN	15430	36	M	1200	150000	90	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Ly-
9	YUVANESH	29934	23	M	32000	30000	90	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Ly-
10	MEHARBANU	60887	32	F	35400	50000	80	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	Ly+
11	ROSELIN MARY	94556	43	F	53400	54000	90	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Ly-
12	THUKKAN	66993	28	M	17000	17000	80	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	Ly-
13	MURALI	6698	22	M	228100	56000	90	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Ly+
14	BANUMATHI	7034	53	F	43000	5000	70	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	Ly-
15	SARITHA	8992	46	F	22700	17000	75	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	Ly-
16	ELUMALAI	93709	46	M	60800	44000	80	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	Ly-
17	ANAND	12006	50	M	60800	19000	70	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	Ly-
18	MANI	50102	50	M	16700	39000	70	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	Ly-
19	PRAVEENA	76548	26	F	4500	19000	25	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	Ly-

NO	NAME	IP NO	AGE	SEX	TOTAL WBC COUNT	PLATELET COUNT	BLAST %	CD3	CD7	CD10	CD13	CD14	CD15	CD19	CD33	CD34	CD 117	Ly
20	SRIRAJ	72793	18	M	44600	77000	60	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	Ly-
21	NAGAIAH	72806	56	M	9000	413000	40	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	Ly-
22	ARULRAJ	82913	33	M	67200	100000	40	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	Ly+
23	SRINIVASAN	60769	32	M	7000	106000	40	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	Ly+
24	KANNAN	59465	66	M	274800	63000	90	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Ly -
25	SHAMEEM	67775	56	M	24000	134000	80	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	Ly -
26	NAGABOOSHANAM	45467	50	F	28200	24000	70	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	Ly+
27	LATHA	98302	22	F	22100	315000	60	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	Ly+
28	KANNIYAPPAN	50841	80	M	27200	136000	70	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	Ly+
29	POWNAMMAL	83245	55	F	29300	486000	30	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	Ly+
30	MANIMARAN	77494	35	M	235600	45000	90	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	Ly -
31	MUNIYAPPAN	50616	45	M	40000	20000	45	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	Ly+
32	RADHI DEVI	70989	40	F	30000	26000	40	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	Ly+
33	SUBRAMANIAM	82334	55	M	18000	15000	55	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Ly -
34	SHYAMALA	54878	43	M	24000	18000	55	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	Ly -
35	DHANALAKSHMI	69043	40	F	10000	25000	40	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Ly+

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.A.Thelengana,
Postgraduate in MD General Medicine,
Institute of Internal Medicine,
Madras Medical College, Chennai-3.

Dear **Dr.A.Thelengana,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "**Aberrant Phenotypes in Acute Myeloid Leukemia in India**" No.13022014.

The following members of Ethics Committee were present in the meeting held on 04.02.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2.	Prof. B.Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3.	Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4.	Prof.Geetha Devadas Professor of Pathology, MMC, Ch-3	-- Member
5.	Prof.K.Sivasubramanian, I/c. Director, Institute of Internal Medicine, MMC, Ch-3	-- Member
6.	Thiru. S. Govindasamy, BA., BL	-- Lawyer
7.	Tmt.Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

13/2/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease, presenting with a high diversity of phenotypes . Immunophenotype in acute myeloid leukemia (AML) had remained elusive. In recent years, along with the wide application of AML immunophenotype testing, immunophenotype itself and its relationship with genetics and morphology become better understood.

The latest WHO 2008 classification of acute leukemia uses morphology, immunophenotype, genetics and clinical features to define clinically significant disease entities . Distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) is extremely important and flow cytometry (FCM) is very instrumental in this. Malignant blasts often have an abnormal phenotype that allows distinction from normal immature cells .

Aberrant phenotype is a well known phenomenon in acute myeloid leukemia. Currently, the aberrant phenotypes are classified into different types: co-expression of lymphoid-associated antigens or lineage infidelity, asynchronous antigen expression, in which early antigens are coexpressed with more mature ones, or

1